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Minireview I

TRANSGENIC RABBITS - *PRODUCTION AND APPLICATION*

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ABSTRACT

Many reports on the production of transgenic rabbits have been directed towards using the rabbit as a model for large domestic animals or as a basic biological model for the study of regulation of mammalian genes. Although, the first transgenic rabbit was produced more than 30 years ago, there are still many factors limiting the efficiency of transgenesis. This minireview summarizes recent research based on the transgenic rabbit model for the transgene integration and expression.

Key words: rabbit, transgene, integration, expression,

INTRODUCTION

Transgenic rabbit production

The rabbit as both a laboratory and domestic animal species provides several opportunities for investigators to study the mechanisms of human disease. Rabbits have the advantage over other large laboratory species in that they have a short gestation period and yield large numbers of embryos. Many reports on the production of transgenic rabbits have been directed towards using the rabbit as a model for large domestic animals or as a basic biological model. Transgenic rabbit is also taken as a temporal biological model between small laboratory organisms (such as mouse) and livestock. And in contrast to mice, rabbits facilitate monitoring of physiological changes without lethal effects. The immediate body size of rabbits between laboratory rodents and domestic animals is favourable for surgical and transplantation studies, for example, lung (Yoshida et al., 2005), heart (Furukawa et al., 2005), bone (Li and Li, 2005), and hepatocytes (Attaran *et al.,* 2004).

Rabbits are famed for their reproductive capabilities. Although certainly not the strongest, fastest, or smartest of the mammals, they have carved out a strong ecological niche through their rate of impregnation, due to the fact that female rabbits ovulate at the time of copulation. Rabbit placentae allows an unusually high degree of contact between maternal and fetal bloodstreams, a condition they share with humans. Thus, they are useful models for the study of human pregnancy and fetal development (Banks, 1989). Therefore, from the viewpoint of reproductive properties rabbit belongs to one of the most suitable biological models for genetic manipulations on embryos.

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Biological advantages of rabbits for manipulations are following:

- provocated ovulation,
- high ovulation coefficient (an average 25 eggs per superovulation),
- short generation interval (short duration of pregnancy, about 30 days, ability to generate a large number of transgenic founders due to a low cost of rabbit embryos),
- sexual maturity in 4-6 months age,
- good pronucleus visualization,
- good embryo survival in *in vitro* conditions
- simple manipulation, (embryotransfer, insemination, etc.),
- as an intermediate between mouse and livestock in the field of transgenesis,
- genetically closer to human than other dairy animals (human therapeutic protein production, alternative model for studying several human disorders),
- mammary gland - post-translation modification (glycosylation, sulfation and acylation) of recombinant proteins,
- rabbit milk, from 150 to 250 ml (depend on peak of lactation) of milk per day of lactation from one female, about 10 liters per year,
- no known prion disease in rabbit and no serious viral disease transmission to human.

The first transgenic rabbit was obtained two decades ago (Hammer et al*.,* 1985; Brem et al*.,* 1985) and some factors influencing the efficiency of rabbit transgenesis have been addressed (Chrenek et al., 1998; 2005, Hirabayashi et al., 2000, 2001; Murakami et al*.,* 2002). The efficiency of transgenic rabbit production is low, ranging from 0.3 to 2.5%. In particular, problems such as low pregnancy rate, small litter size, cannibalism, mosaicism and low transgene transmission rates have been observed.

One of important factors limiting the efficiency of the production of transgenic rabbits is the low rate of transgene incorporation into the genome of microinjected embryos. Another important factor is the stability of transgene transmission to offspring. Several attempts have been made to improve the efficiency of genomic integration of foreign DNA. Page et al. (1995) attempted to produce transgenic mice by cytoplasmic injection of DNA mixed with polylysine. Seo et al. (2000) doubled the efficiency of transgenesis by co-injecting restriction endonuclease together with foreign DNA into mouse pronuclei. Hirabayashi et al. (2000) used zygote centrifugation to visualize pronuclei and produce transgenic rabbits. The effect of DNA concentration on the rate of transgenesis was also tested (Nottle et *al.,* 2001). Transgenic rabbits have also been produced using sperm-mediated gene transfer, however, low expression and rearrangement of the transgene was observed (Kuznetsov et al., 2000). Lipofectin-mediated gene transfer via sperm led to the production of transgenic rabbits (Wang et al., 2001). A double pronuclear microinjection (DM) technique was successfully used to improve the production of transgenic mice (Kupriyanov et al., 1998) and transgenic rabbits (Chrenek et al., 2005).

The overall efficiency of transgenic rabbit production per injected zygote ranges from 0.3% to 4.2%. Transgene integration efficiency in rabbits has been reported to range between 2% and 31%, depending on the gene construct and its concentration (Table 1). Hammer et al., (1985) reported a 13% integration efficiency of the hGH gene into the rabbit genome, Snyder et al., (1995) about 18% with the hCD4 gene, Hirabayashi et al., (2000) a 4-8% efficiency with the hGH gene, Murakami et al., (2002) a 31% efficiency with hCD55 gene, Chrenek et al., (2002) 3% with the hPC gene, Hiripi et al. (2003) about 2% with hFVIII-Mt gene and Lipinski et al., (2003) about 4.5% with the hGH gene. It is possible that the increase in the amount of DNA, by microinjection of transgene into both pronuclei (Kupriyanov et al., 1998, Chrenek et al., 2005), increases the probability of integration into the genome, as compared to single microinjection. This point of view is supported by a previous report, where the injection into bovine embryos of a higher concentration of EGFP gene, 8.0 ng/µl versus 4.8 ng/µl, increased the expression of EGFP at the blastocyst stage (Murakami et al., 2003). Using the same DNA volume and concentration for both hFVIII and EGFP constructs (5ng/µl), a similar survival rate was obtained for rabbit embryos and newborn rabbits.

Table 1: Efficiency of transgenic rabbit production

Gene construct	Efficiency of gene Integration (%)	Reference
mWAP-hPC	0.5-3.0	Chrenek et al., 2002
WAP-hFVIII-Mt-I	2.0	Hiripi et al., 2003
mWAP-hFVIII	1.6-3.1	Chrenek et al., 2005
Tg(*Wap-GH1)*	*1.2*	Skrzyszowska et al., 2006
CMVIE/EGFP	17.0	Chrenek et al., 2005
CAG/EGFP	0.75	Takahashi et al., 2007

Voss et al. (1990) evaluated factors decreasing the efficiency: species of zygotes, varying intensities of microscope light, different type of injection pipettes, and different genes tested for their influence on the efficiency of pronuclear gene injection for the production of transgenic rabbits and mice. They found that rabbit zygotes were less sensitive to mechanical manipulation during injection than mouse zygotes and exposure of zygotes to a microscope light intensity of 5550 lux

significantly reduced their cleavage rate, while a lower intensity (2280 lux) did not; implantation rates also varied between 2.9% and 23.1% depending on the gene used. Murakami and co-workers (2002) suggested that the gene construct and the survival rate of injected embryos are important factors affecting the efficiency of producing transgenic rabbits, and the age of recipients is also one of the important factors affecting the survival rate of the injected embryos. Popova et al. (2004) studied the factors affecting the efficiency of transgenic technology in rats and concluded that the main detrimental factor in the microinjection of rat zygotes is the introduction of buffer solution into the pronucleus. They also stated that overnight culture of zygotes between microinjection and oviduct transfer does not increase the efficiency of transgenic rat generation. Further, the majority of transgenic animals generated by pronuclear microinjection appears to be mosaic in both somatic cells and germ cells for the pattern of DNA integration (Wall, 1996). This could be caused by late integration of transgenes during embryonic preimplantation development (Wall and Seidel, 1992). Chrenek et al. (1998), while studying the factors influencing the developmental potential of cultured rabbit zygotes observed that the hormonal treatment of rabbit donors resulted in a doubling of the number of recovered ova per donor when compared with the non-treated group. However, the quality of recovered zygotes (presence of both pronuclei) in hormonally treated group was significantly worse. In other studies, Chrenek and co-workers (1999, 2005) successfully used superovulation to increase the embryo yield and they also reported that double microinjection of DNA (into both pronuclei of rabbit embryos) is more efficient (in regards to transgene integration) to produce a large number of transgenic animals than single microinjection. Makarevich et al. (2005) suggested that apoptosis is one of the major causes of the low quality and viability of microinjected embryo, as microinjection-derived cleavage-arrested embryos exhibited a poorly developed nucleolus, swollen mitochondria, several lipid vesicles, an extensive area with dispersed electron-dense material as well as nuclear membrane blebbing and numerous electron-dense bodies. Other general factors believed to influence the production of transgenic rabbits include:

a. breed
b. availability of fertilized eggs for microinjection
c. evaluation of obtained eggs
d. culture of embryos
e. microinjection of eggs
f. embryo transfer

In association with the gene construct, the choice of promoters is very important for the success of a particular transgenesis experiment. Constitutive promoters direct the expression of genes in almost all tissues and are independent of any environmental or developmental conditions. Besides constitutive promoters, tissue-specific promoters and inducible promoters can also be used for transgenesis.

Use of transgenic rabbits

The transgenic rabbits provides opportunities for the study of several processes

■ lipid metabolism and atherosclerosis

Transgenic rabbits expressing human apolipoprotein ‘‘apo A’’ were produced with the aim of revealing its relationship with apolipoprotein ‘‘apo B’’ forming Lp(a) complex (Fan et al., 2001). These studies provide new insights into the mechanisms responsible for the development of atherosclerosis, emphasizing the strength of the rabbit model in cardiovascular disease research.

■ cardiovascular functions

Transgenic rabbits as model for studies of cardiovascular functions were successfully obtained with subsequent expression of mutated human β-myosin heavy chain. Clinical symptoms (such as hypertrophy of heart, interstitial fibrosis etc.) accounted for the symptoms of treated patients (Marian et al., 1999).

■ viral diseases

Experimental rabbits are essential for the development of protective and defensive agents against viral diseases. Typical example is Acquired Immuno-Deficiency Syndrome (AIDS). Laboratory rabbits are easily contaminated by HIV. Due to their sensitivity to HIV-1 they are preferred for research in this field. They are also characterized by low progress of infection in comparison to human being, which is brought about by the differences between human and rabbit CD4 linkage sites on viral protein HIV gp120. For that purpose transgenic rabbits expressing human CD4 (Snyder et al., 1995) were produced.

■ oncogenic diseases

Transgenic rabbits showed symptoms of leukemia even before reaching sexual maturity. Later, tumors were reported and confirmed on ovaries and basal cells (Knight et al., 1988). Further studies relating to the uses of these transgenic rabbits for clinical studies or pharmacological testing will be of considerable significance.

■ anatomical, metabolic and histological pathology

In fact, the first transgenic rabbits, which were recovered with gene for GH contained several promoters (Hammer et al., 1985; Brem et al., 1985). Problems with

expression of GH were manifested mainly in fertility and libido in transgenic males. New gene constructs have enhanced the success of utilizing transgenic rabbits for such purposes. There exists a real assumption that transgenic rabbit would be a suitable model for osteroarthritidis (Fernandes et al., 1999).

■ production of recombinant proteins

Transgenic rabbit is an alternative model for the production of therapeutic proteins in milk (Fan and Watanabe, 1999, 2003, Bozse et al., 2003), especially those, that are required in smaller amounts. Generally, transgenic animals present an alternative in biologically active protein production (Paleyanda, 1997). Recombinant proteins can be produced in prokaryotic or eukaryotic systems, so that they are derived from bacterial cells, yeast cells, transformed plant and animal cells, and even from live bioreactors (transgenic animals). Difficulty in obtaining transgenic individuals producing high levels of recombinant, biologically active proteins in required form, with subsequent washing and clinical testing are the largest disadvantages of using farm animals for such purposes. Lower financial expenditure for obtaining transgenic individuals, suitable reproductive properties, and also lower cost for maintaining transgenic generations are the causes for preference of rabbits over other farm animals.

Rabbit is the smallest domestic animal which can be utilized for production of recombinant proteins for experimental and commercial purposes. It is an alternative model for the production of therapeutic proteins in milk (Fan and Watanabe, 2003, Bozse et al., 2003), especially those that are required in smaller amounts. While mouse is a good model for leading experiments, mainly with the aim of testing new gene constructs (integration and expression), rabbits can produce up to 50 ml of milk daily. Production of several thousands kilograms of milk per year can be achieved from dairy cattle, in cases several hundreds of kilograms from sheep and goat, but from rabbits only several kilograms per year. Rabbit milk, however, contains 2.5 times higher protein than sheep milk and 4.8 times higher than the goat milk (Jennes, 1974). Still rabbits are good candidates for the expression of human genes in their lactating mammary gland because they can effectively process complex proteins as they can express tens to hundreds grams of such proteins in their milk during lactation. Castro and co-workers (1999) have discussed the potential use of rabbits as bioreactors in their work. Advantage for the production of pharmaceutical proteins using transgenic rabbits is that, rabbits may be reared in specific pathogen free conditions at lower costs. The same conditions may be useful also for human diseases research. Medicinal products from transgenic rabbits (Table 2), which are available, may be divided into following three groups:

a. monoclonal antibodies (e.g. mouse monoclonal antibody,…)
b. hormones and bioactive peptides (e.g. IGF-1,.)
c. therapeutic proteins (e.g. hPC, hFVIII,…)

Table 2: Therapeutic proteins produced by transgenic rabbit mammary gland

Recombinant protein	Aplication	Reference
h GH	Insufficient GH	Hammer et al., 1985
H alfa1-antitrypsin	Emphysema	Massoud et al., 1990
h tPA	Thrombosis	Riego et al., 1993
H IGF-1	Insufficient GH	Brem and Muller, 1994
Bochymosine	Cheese production	Brem et al., 1995
H erythropoietin	Anaemia	Rodriguez et al., 1995
H ESDM	Ischemy	Stromqvist et al., 1997
salmon calcitonin	Osteoporosis	McKee et al., 1998
H alfa-glucosidase	Glycogen	Bijvoet et al., 1999
h NGF-beta	Neuropathy	Coulibaly et al., 1999
h PC	Insufficient hPC	Chrenek et al., 2002
hFVIII	Hemophilia A	Hiripi et al., 2003
hFVIII	Hemophilia A	Chrenek et al., 2005

Table 3: Production of recombinant human FVIII using different animals and gene constructs

Animal	Gene construct	Concentration of rhFVIII	Reference
Mouse	αLA-hFVIII	50.2μg/ml	Chen et al., 2002
Rabbit	WAP-hFVIII-Mt-I	-	Hiripi et al., 2003
Rabbit	WAP-hFVIII	1.2mg/ml	Chrenek et al., 2005
Sheep	beta-Lac–hFVIII-Mt-I	6ng/ml	Nieman et al., 1999
Pig	WAP-hFVIII	2.7μg/ml	Paleyanda et al., 1997

The first transgenic rabbits as bioreactors bearing the fusion gene for the production of human growth hormone were obtained in mid 1990s (Limnota et al., 1995). Szalata and co-workers (2004) purified and evaluated biologically active human growth hormone produced in the mammary gland of transgenic rabbits bearing a transgene on chromosome 7q, which was shown to exert no influence on transgenic animals.

Many reports on the production of transgenic rabbits have been directed towards using the rabbit as a model for large domestic animals or as a basic biological model for the study of regulation of mammalian genes (Sarda et al., 2002). Transgenic rabbit may also be an important bioreactor for the production of various pharmaceutical proteins (Castro et al., 1999; Chrenek et al., 2002; 2005). It is an alternative model for the production of therapeutic proteins in milk (Fan et al., 1999; Fan and Watanabe, 2003; Bozse et al., 2003), especially those that are required in smaller amounts. In fact, rabbits were the first animal model of artherosclerosis (Clarkson et al., 1974). It continues to be an excellent model for the study of lipoprotein metabolism, hypertrophic cardiography and artherosclerosis, emphasizing the strength of the rabbit model in cardiovascular disease research (Brousseau and Hoeg, 1999). However, the most controversial and yet promising aspect of the technology involves the "selective improvement" of species by the modification of the genome, that is, modification of animal anatomy and physiology. Transgenic rabbits offer an attractive alternative in this field to other large dairy animals in that they have a large litter size and short generation interval (Dove, 2000; Hiripi et al., 2003) accompanied by short gestation period and yield large numbers of embryos. This species avoids some of the disadvantages of large animals, such as pigs or sheep, and small animals, such as mice. In spite of more than 20 years of research by numerous investigators the success of transgenic rabbit production is limited (2-3%). This necessitates modification of the existing techniques or, for instance, development of new techniques for efficient production of transgenic rabbits. In future, other products including novel therapeutic proteins and glycoproteins, as well as vaccines, will be produced in milk, blood or other products of biopharm animals including rabbits.

For the production of recombinant therapeutic proteins in transgenic animals the stability of transgene transmission over multiple generations in multiple lines is crucial (Van Cott et al., 1997). Extensive studies of the effects of recombinant protein on animals itself and on several generations must be performed before setting up a herd for production purposes (Lubon et al. 1996).

Recombinant human factor VIII (hFVIII) production

To express hFVIII protein, different mammary gland specific hFVIII gene constructs have previously been used in different animals (Table 3).

Transgenic pigs, sheep, rabbits and mice have been generated and variable levels of rhFVIII expression were obtained depending on the gene regulatory sequences used. Usage of a construct consisting of the hFVIII cDNA directed by mouse WAP promotor resulted in transgenic pigs expressing rhFVIII (Paleyanda et al., 1997), a gene construct consisting of ovine β-lactoglobulin (B-LG)-hFVIII cDNA led to low level expression in transgenic sheep (Niemann et al., 1999), whereas a bovine α-lactalbumin (A-LA)-hFVIII-bGHp(A) gene construct expressed rhFVIII at higher levels in transgenic mice (Chen et al., 2002). Our transgenic rabbit founders derived using the mouse WAP-hFVIII cDNA construct also expressed higher levels of rhFVIII (Chrenek et al., 2005), than transgenic pigs or transgenic rabbits with the mWAP-hFVIII-MT1 gene construct (Hiripi et al., 2003). There are, however, many factors which may negatively influence the level of rhFVIII activity, such as incomplete glycosylation. N-linked oligosacharide in 25 potential sites within hFVIII polypeptide sequences is necessary to build the complex - type of tertiary structure (Dorner et al., 1987). The second reason might be unsuccessful processing of factors that are required for post-translational

modifications (Chen et al., 2002). Low clotting activity of rhFVIII could be also due to the instability of the rhFVIII in the rabbit milk lacking von Willebrand factor (vWF) as a carrier protein. A FVIII–vWF complex formation might prevent premature binding of factor VIII to components of the factor X activating complex. It is also possible that rhFVIII, produced in rabbit mammary gland interacting with rabbit milk components, such as casein micelles or lipids are reorganized by the antibodies in the ELISA assay as explained previously by Paleyanda et al. (1997) in transgenic pigs. The genetic and in vitro stability of rhFVIII may also influence final quality during purification, dilution and analyses (Parti et al., 2000). Western-blots revealed that single rhFVIII chain content was dependent on the expression level and varied between transgenic rabbit females. This may suggest, as reported in transgenic pigs (Van Cott et al., 1997), that rabbit genetics may play a role in selection of productive lines of rabbits with optimal post-translational proteolytic processing capability.

Although rabbits are not conventional dairy livestock, it is agreed that the short generation time, multiple offspring per litter, stable patternal transmission of the transgene and milk yield offer advantages over conventional dairy livestock for the establishment of a line producing a therapeutic recombinant protein in sufficient concentration and biological activity. Since a high variability between generations and individual transgenic rabbits in rhFVIII concentration and biological activity is observed, an individual evaluation and selection of transgenic animals for commercial use is required.

REFERENCES

ATTARAN, M. – SCHNEIDER, A. – GROTE, C. – ZWIENS, C. – FLEMMIN, G. P. – GRATZ, K.F. – JOCHHEIM, A. – BAHR, M.J. – MANNS, M.P. – OTT, M. 2004. Regional and transient ischemia/reperfusion injury in the liver improves therapeutic efficacy of allogeneic intraportal hepatocyte transplantation in low-density lipoprotein receptor deficient Watanabe rabbits. In: *J. Hepatol.,* vol. 41(5), 2004, p. 837-844.

BANKS, R. 1989. Rabbits: Models and Research Applications (USAMRIID Seminar Series)“ (On-line). http://netvet.wustl.edu/species/rabbits/rabtmodl.txt.

BIJVOET, A.G. - VAN HIRTUM, H. – KROOS, M. A. - VAN DE KAMP, E. H. – SCHONEVELD, O. – VISSER, P. – BRAKENHOFF, J. P. – WEGGEMAN, M. - VAN CORVEN, E. J. - VAN DER PLOEG, A. T. – REUSER, A. J. 1999. Human acid alfa-glucosidase from rabbit milk has therapeutic effect in mice with glycogen storage disease type II. In: *Human Mol. Genet.,* vol. 7, 1999, p. 2145-2153.

BOZSE, Z. – HIRIPI, L. – CARNWATH, J. W. – NIEMAN, H. 2003. The transgenic rabbit as model for human diseases and as a source of biologically active recombinant proteins. In: *Transgenic Res.,* vol. 12, 2003, p. 541-553.

BREM, G. – BESENFELDER, U. – ZINOVIEVA, N. – SEREGI, J. – SOLTI, L. – HARTL, P. 1995. Mammary gland specific expression of chymosin constructs in transgenic rabbits. In: *Theriogenology,* vol. 43, 1995, p.175.

BREM, G. – BRENING, B. – GOODMAN, H. M. – SELDEN, R. C. – GRAF, F. – KRUFF, B. – SPRINGMAN, K. – HONDELE, J. – MEYER, J. – WINNACKER, E. L. – KRAUSSLICH, H. 1985. Production of transgenic mice, rabbits and pigs by microinjection into pronuclei. In: *Zuchthygiene,* vol. 20, 1985, p. 251-252.

BREM, G. – MULLER, M. 1994. Large transgenic animals. In: MacLean N, editor. Animal with novel genes. Cambridge, England: Cambridge University Press., 1994, p.179-244.

BROUSSEAU, M.E. – HOEG, J. M. 1999. Transgenic models as models for atherosclerosis research. In: *J. Lipid Research,* vol. 40, 1999, p. 365-375.

BUHLER, T. A. – BRUYERE, T. – WENT, D. F. – STRANZINGER, G. – BURKI, K. 1990. Rabbit β-casein promoter directs secretion of human interleukin-2 into the milk of transgenic rabbits. In: *Biol. Tech.,* vol. 8, 1990, p. 140-143.

CASTRO, F. O. – LIMONTA, J. – RODRIQUEZ, A. – AGUIRRE, A. - DE LA FUENTE, J. – AGUILAR, A. – RAMOS, B. – HAYES, O. 1999. Transgenic rabbits for the production of biologically active recombinant proteins in the milk. In. *Genet. Anal.*, vol. 15(3-5), 1999, p. 179-187.

CHEN, S. H. – VAUGHT, T. D. – MONAHAN, J. A. – BOONE, J. – EMSLIE, E. – JOBST, P. M. – LAMBORN, A. E. – SCHNIEKE, A. – ROBERTSON, L. – COLMAN, A. – DAI, Y. – POLEJAEVA, A. – AYARES, D. L. 2002. Efficient production of transgenic cloned calves using preimplantation screening. In: *Biol. Reprod.,* vol. 67, 2002, p. 1488-1492.

CHRENEK, P. – MAKAREVICH, A. V. – VASICEK, D. – LAURINCIK, J. – BULLA, J. – RAFAY, J. 1998. Effects of superovulation, culture and microinjection on development of rabbit embryos *in vitro*. In: *Theriogenology,* vol. 50, 1998, p. 659-666.

CHRENEK, P. – PETROVICOVA, I. – RAFAY, J. – BULLA, J. 1999. Superovulation and recovery of zygotes suitable for double-microinjection in three rabbit populations. In: *Czech J. Anim. Sci.,* vol. 44, 1999, p. 471-474.

CHRENEK, P. – VASICEK, D. – MAKAREVICH, A. V. – JURCIK, R. – SUVEGOVA, K. – PARKANYI, V. – BAUER, M. – RAFAY, J. – BATOROVA, A. – PALEYANDA, R. K. 2005. Increased transgene integration efficiency upon microinjection of DNA into both pronuclei of rabbit embryos. In: *Transgenic Res.,* vol. 14, 2005, p. 417-428.

CHRENEK, P. – VASICEK, D. – MAKAREVICH, A. V. – UHRIN, P. – PETROVICOVA, I. – LUBON, H. – BINDER, B. R. – BULLA, J. 2002. Integration and expression of the WAP-hPC gene in three generations of transgenic rabbits. In: *Czech J. Anim. Sci.,* vol. 47(2), 2002, p. 44-49.

CLARKSON, T. B. – LEHNER, N. D. M. – BULLOCK, B. C. 1974. Specialized research applications: I.Atherosclerosis research. In: *The Biology of the Laboratory Rabbit* (Weisbroth SH, Flatt RE, and Krauss AL, Eds.), Academic Press, New York, 1974, p.155-165.

COULIBALY, S. – BESENFELDER, U. – FLEISCHMANN, M. – ZINOVIEVA, N. – GROSSMANN, A. – WOZNY, M. – BARTKE, I. – TOGEL, M. – MULLER, M. – BREM, G.

1999. Human nerve growth factor beta (hNGF-β): mammary gland specific expression and production in transgenic rabbits. In: *FEBS Lett.,* vol. 444, 1999, p. 111-116.

DORNER, A. J. – BOLE, D. G. – KAUFMAN, R. J. 1987. The relationship of N-linked glycosylation and heavy chain-binding protein association with the secretion of glycoproteins. In: *J. Cell Biol.,* vol. 105, 1987, p. 2665-2674.

DOVE, A. 2000. Milking the genome for profit. In: *Nature Bitech.,* vol. 18, 2000, p. 1045-1048.

FAN, J. – CHALLAH, M. – WATANABE, T. 1999. Transgenic rabbit model for biomedical research: Current status, basic methods and future perspectives. In. *Pathol. Int.,* vol. 49, 1999, p. 583-594.

FAN, J. – SUN, H. – UNOKI, H. – SHIOMI, M. – WATANABE, T. 2001. Enhanced atherosclerosis in Lp(a) WHHL transgenic rabbits.In: *Ann. NY. Acad. Sci.,* vol. 947, 2001, p. 362-365.

FAN, J. – WATANABE, T. 2003. Transgenic rabbits as therapeutic protein bioreactors and human disease models. In: *Pharmacol. Therapeut.,* vol. 99, 2003, p. 261-282.

FERNANDES, J. – TARDIF, G. - MARTEL-PELLETIER, J. - LASCAU-COMAN, V. – DUPUIS, M. – MOLDOVAN, F. 1999. *In vivo* transfer of interleukin-1 receptor antagonist gene in osteoarthritic rabbit knee joints: prevention of osteoarthritis progression. In: *Am. J. Pathol.,* vol. 154, 1999, p. 1159-1169.

FURUKAWA, H. – OSHIMA, K. – TUNG, T. – CUI, G. – LAKS, H. – SEN, L. 2005. Liposome-mediated combinatorial cytokine gene therapy induces localized synergistic immunosuppression and promotes long-term survival of cardiac allografts. In: *J. Immunol.,* vol. 174(11), 2005, p. 6983-6992.

HAMMER, R. E. – PURSEL, V. G. – REXROAD, C. E. – WALL, R. J. – BOLT, D. J. – EBERT, K. M. 1985. Production of transgenic rabbits, sheep and pigs by microinjection. In: *Nature,* vol. 315, 1985, p. 680-683.

HIRABAYASHI, M. – HIRAO, M. – TAKAHASHI, R. – KIMURA, K. – HIRASAWA, K. – UEDA, M. 2000. Production of transgenic rabbits using centrifuged pronuclear zygotes. In: *J. Vet. Med. Sci.,* vol. 62, 2000, p. 1047-1052.

HIRABAYASHI, M. – TAKAHASHI, R. – ITO, K. – KASHIWAZAKI, N. – HIRAO, M. –HIRASAWA, K. 2001. A comparative study on the integration of exogenous DNA into mouse, rat, rabbit and pig genomes. In: *Exp. Anim.,* vol. 50, 2001, p. 125-131.

HIRIPI, L. – MAKOVICS, F. – HALTER, R. – BARANYI, M. – PAUL, D. – CARNWATH, J. W. – BOSZE, Z. – NIEMANN, H. 2003. Expression of active human blood clotting factor VIII in the mammary gland of transgenic rabbits. In: *DNA Cell Biol.,* vol. 22, 2003, p. 41-45.

JENNES, R. 1974. The composition of milk. In: *Lactation: A Comprehensive Treatise*. Academic Press, New York, 1974. p. 3-105.

JESSEN, J. R. - WILLETT, C. E. - LIN, S. 1999. Artificial chromosome transgenesis reveals long-distance negative regulation of rag1 in zebrafish. In: *Nat. Genet.,* vol. 23, 1999, p. 15-16.

KNIGHT, K. L. - SPIEKER-POLET, H. – KAZDIN, D. S. – OI, V. T. 1988. Transgenic rabbits with lymphocytic leukemia induced by the c-myc oncogene fused with the immunoglobulin heavy chain enhancer. In: *Proc. Natl. Acad. Sci. USA,* vol. 85(9), 1988, p. 3130-3134.

KUPRIYANOV, S. – ZEH, K. – BARIBAULT, H. 1998. Double pronuclei injection of DNA into zygotes increase yields of transgenic mouse lines. In: *Transgenic Res.,* vol. 7, 1998, p. 223-226.

KUZNETSOV, A. V. – KUZNETSOV, I. V. – SCHIT, I. Y. 2000. DNA interaction with rabbit sperm cells and its transfer into ova *in vitro* and *in vivo*. In: *Mol. Reprod. Dev.,* vol. 56, 2000, p. 292-297.

LI, Z. – LI, Z. B. 2005. Repair of mandible defect with tissue engineering bone in rabbits. In: *J. Surg.,* vol. 75(11), 2005, p. 1017-1021.

LIMNOTA, J. M. – CASTRO, F. O. – MARTINEZ, R. – PUENTES, P. – RAMOS, B. – AGUILAR, A. – LEONART, R. L. - DE LA FUENTE, J. 1995. Transgenic rabbits as bioreactors for the production of human growth hormone. In: *J. Biotechnol.,* vol. 40, 1995, p. 49-58.

LIPINSKI, D. – JURA, J. – KALAK, R. – PLAWSKI, A. – KALA, M. – SZALATA, M. – JARMUZ, M. – KORCZ, A. – SLOMSKA, K. – JURA, J. – GRONEK, P. – SMORAG, Z. – PIENKOWSKI, M. – SLOMSKI, R. 2003. Transgenic rabbit producing human growth hormone in milk. In: *J. Appl. Genet.,* vol. 44, 2003, p. 165-174.

LUBON, H. – PALEYANDA, R. K. – VELANDER, W. H. – DROHAN, W. N. 1996. Blood proteins from transgenic animal bioreactors. In: *Transfusion Med. Reviews*., vol. 10(2), 1996, p. 131-143.

MAKAREVICH, A. V. – CHRENEK, P. – ZILKA, N. – PIVKO, J. – BULLA, J. 2005. Preimplantation development and viability of in vitro cultured rabbit embryos derived from in vivo fertilized gene-microinjected eggs: apoptosis and ultrastructure analyses. In: *Zygote,* vol. 13, 2005, p. 125-137.

MARIAN, A. J. – WU, Y. – LIM, D. S. 1999. A transgenic rabbit model for human hypertrophic cardiomyopathy. In: *J. Clin. Invest.,* vol. 104, 1999, p. 1683-1692.

MASSOUD, M. – BISCHOFF, R. – DALEMANS, W. – POINTU, H. – ATTAL, J. – SCHULTZ, H. – CLESSE, D. – STINNAKRE, M. G. – PAVIRANI, A. – HOUDEBINE, L. M. 1990. The production of human proteins in the blood of transgenic animals. In: *C. R. Acad. Sci.,* vol. 311(8), 1990, p. 275-280.

MCKEE, C. – GIBSON, A. – DALRYMPLE, M. – EMSLIE, L. – GARNER, I. – COTTINGHAM, I. 1998. Production of biologically active salmon calcitonin in the milk of transgenic rabbits. In: Nat. Biotech., vol. 16, 1998, p. 647 – 651.

MURAKAMI, H. – FUJIMURA, T. – NOMURA, K. – IMAI, H. 2002. Factors influencing efficient production of transgenic rabbits. In: *Theriogenology, v*ol. 57(9), 2002, p. 2237-2245.

MURAKAMI, M. – IDEGUCHI, S. – FAHRUDIN, M. – OTOI, T. – GODKE, R. A – SUZUKI, T. 2003. Influence of the DNA amount per microinjection on the development and EGFP expression in bovine embryos. In: *Arch Tierz Dummerstorf,* vol. 46 (1), 2003, p. 25-30.

NIEMANN, H. – HALTER, R. – CARNWATH, J. W. – HERRMANN, D. – LEMME, E. – PAUL, D. 1999. Expression of human blood clotting factor VIII in the

mammary gland of transgenic sheep. In: *Transgenic Res.,* vol. 8, 1999, p. 137-149.

NOTTLE, M. B. – HASKARD, K. A. – VERMA, P. J. – DU, Z. T. – GRUPEN, C. G. – MCILFATRICK, S. M. – ASHMAN, R. J. – HARRISON, S. J. – BARLOW, H. – WIGLEY, P. L. – LYONS, I. G. – COWAN, P. J. – CRAWFORD, R. J. – TOLSTOSHEV, P. L. – PEARSE, M. J. – ROBINS, A. J. - D'APICE, A. J. 2001. Effect of DNA concentration on transgenesis rates in mice and pigs. In: *Transgenic Res*., vol. 10(6), 2001, p. 523-31.

PAGE, R. L. – BUTLER, S. P. – SUBRAMANIAN, A. – GWAZDAUSKAS, F. C. – JOHNSON, J. L. – VELANDER, W. H. 1995. Transgenesis in mice by cytoplasmic injection of polylysine/DNA mixtures. In: *Transgenic Res.,* vol. 4, 1995, p. 353-360.

PALEYANDA, R. K. – VELANDER, W. H. – LEE, T. K. – SCANDELLA, D. H. – GWAZDAUSKAS, F. G. – KNIGHT, J. W. – HOYER, L. W. – DROHAN, W. N. – LUBON, H. 1997. Transgenic pigs produce functional human factor VIII in milk. In: *Nat. Biotech.,* vol. 15(10), 1997, p. 971-975.

PARTI, R. – ARDOSA, J. – YANG, L. – MANKARIOUS, S. 2000. In vitro stability of recombinant human factor VIII (Recombinate). In: *Haemophilia,* vol. 6, 2000, p. 513-522.

POPOVA, E. – BADER, M. – KRIVOCHARCHENKO, A. 2004. Strain Differences in Superovulatory Response, Embryo Development and Efficiency of Transgenic Rat Production. In: *Transgenic Res.,* vol. 14(5), 2004, p. 729-738.

RIEGO, E. – LIMNOTA, J. – AGUILAR, A. – PEREZ, A. – ARMAS, D. R. – SOLTANO, R. – RAMOS, B. – CASTRO, F. O. - FUENTE DE LA, J. 1993. Production of transgenic mice and rabbits that carry and express the human tissue plasminogen activator cDNA under the control of a bovine αS1 casein promoter. In: *Theriogenology,* vol. 39, 1993, p. 1173-1185.

RODRIGUEZ, A. – CASTRO, F. O. – AGUILAR, A. – RAMOS, B. - DEL BARCO, D. G. – LEONART, R. - DE LA FUENTE, J. 1995. Expression of active human erythropoietin in the mammary gland of lactating transgenic mice and rabbits.In: *Biol. Res.,* vol. 28, 1995, p. 141-153.

SEO, B. B. – KIM, C. H. – YAMANOUCHI, K. – TAKAHASHI, M. – SAWASAKI, T. – TACHI, C. – TOJO, H. 2000. Co-injection of restriction enzyme with foreign DNA into the pronucleus for elevating production efficiencies of transgenic animals. In: *Anim. Repris. Sci.,* vol. 63, 2000, p. 113-122.

SKRZYSZOWSKA, M. – SMORAG, Z. – SLOMSKI, R. - KATSKA-KSIAZKIEWICZ, L. – KALAK, R. – MICHALAK, E. – WIELGUS, K. – LEHMANN, J. – LIPINSKI, D. – SZALATA, M. – PLAWSKI, A. – SAMIEC, M. – JURA, J. – GAJDA, B. – RYNSKA, B. – PIENKOWSKI, M. 2006. Generation of transgenic rabbits by the novel technique of chimeric somatic cell cloning. In: *Biol. Reprod.,* vol. 104, 2006, p. 1114–1120.

SNYDER, B. W. – VITALE, J. – MILOS, P. – GOSSELIN, J. – GILLESPIE, F. – EBERT, K. 1995. Development and tissue-specific of human CD4 in transgenic rabbits. In: *Mol. Reprod. Dev.,* vol. 40, 1995, p. 419-428.

STROMQVIST, M. – HOUDEBINE, L. M. – ANDERSON, J. O. – EDLUND, A. – JOHANSSON, T. – VIGLIETTA, C. – PUISSANT, C. – HANSSON, L. 1997. Recombinant human extracellular superoxide dismutase produced in milk of transgenic rabbits. In: *Transgenic Res.,* vol. 6, 1997, p. 271-278.

SZALATA, M. – LIPINSKI, D. – KALAK, R. – TOBOLA, P. – LEHMANN, J. – WIELGUS, K. – JURA, J. – SMORAG, Z. – PIENKOWSKI, M. – SLOMSKI, R. 2004. Purification and characterization of the human growth hormone obtained in the milk of transgenic rabbits. In: *Ann. Anim. Sci.,* vol. 4(2), 2004, p. 351-362.

TAKAHASHI, R. – KURAMOCHI, T. – AOYAGI, K. – HASHIMOTO, S. – MIYOSHI, I. – KASAI, N. – HAKAMATA, Y. – KOBAYASHI, E. – UEDA, M. 2007. Establishment and characterization of CAG/EGFP transgenic rabbit line. In: *Transgenic Res.,* vol. 16, 2007, p. 115-120.

VAN COTT, K. E. – LUBON, H. – RUSSEL, C. G. – BUTLER, S. P. – GWAZDAUSKAS, F. C. – KNIGHT, J. 1997. Phenotypic and genotypic stability of multiple lines of transgenic pigs expressing recombinant human protein C. In: *Transgenic Res.,* vol. 6, 1997, p. 203-212.

VOSS, A. K. – SANDMOLLER, A. – SUSKE, G. – STROJEK, R. M. – BEATO, M. – HAHN, J. 1990. A comparison of mouse and rabbit embryos for the production of transgenic animals by pronuclear microinjection. In: *Theriogenology,* vol. 34(5), 1990, p. 813-24.

WALL, R. J. 1996. Transgenic livestock: progress and prospects for the future. In: *Theriogenology,* vol. 45, 1996, p. 57-68.

WALL, R. J. – SEIDEL, G. E. Jr. 1992. Trangenic farm animals: a critical analysis. In: *Theriogenology,* vol. 38, 1992, p. 337-357.

YOSHIDA, S. – SEKINE, Y. – SAITOH, Y. – YASUFUKU, K. – IWATA, T. – FUJISAWA, T. 2005. Surgical technique of experimental lung transplantation in rabbits. In: *Ann. Thorac. Cardiovasc. Surg.,* vol. 11(1), 2005, p. 7-11.

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