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REVIEW

COMPARISON TRANSGENIC AND NON-TRANSGENIC MILK QUALITY

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ABSTRACT

Transgenic founder rabbits carrying a gene construct consisting of a 2.5 kb murine whey acidic protein promoter (mWAP), 7.2 kb of the human clotting factor VIII (hFVIII) cDNA and 4.6 kb of 3' flanking sequences of mWAP gene were crossed for five generations.

Transgenic females showed high level of recombinant hFVIII (rhFVIII) mRNA expression in biopsed mammary gland tissues. The presence of the mWAP-hFVIII transgene in rabbit genome and secretion of rhFVIII into milk of transgenic females (F_1, F_2, F_3, F4 and F5 generation) did not have any adverse phenotypic effect on milk quality.

Keywords: transgenic rabbit, mWAP-hFVIII, milk quality

INTRODUCTION

Mammary gland as a bioreactor

The use of transgenic animals as bioreactor ("pharmaceutical farming" or "gene farming") is a cost-effective and variability in post-translational modification alternative to

cell culture methods. Animals automatically supplement their body fluids with fresh nutrients, remove waste products, reliably regulate their internal temperature and pH and resist to pathogens. By targeting the expression of the transgene product, so that the secretory cells of lactating mammary gland produce it, "farmers" may collect and process body fluids with minimal effort. The mammary gland probably is the most promising target tissue because it produces large amounts of protein in a temperature-regulated fluid that may be collected daily in a non-invasive fashion. Transgenic animals are not only cost-effective bioreactors, but, with the complex secretory cell types and organs of the mammalian organism, can perform much more complicated protein modifications than simply cultured cells. Transgenic animals used as bioreactors to produce human proteins represent new horizon in animal husbandry often followed by low viability of newborn animals. Repercussion of that can be also irregular secretion and alteration of milk composition.

Milk quality and quantity of transgenic animals

Milk is usually the sole source of nourishment of young mammals, therefore offspring growth and development depends only on milk. Rabbit milk yield may be affected by breed of doe (**Lukafahr et al., 1983**), nutrition (**Chrastinova et al., 1997**), number of kids suckling and their age of weaning (**Taranto et al., 2003**) and pregnancy during lactation (**Lukafahr et al., 1983**). Intensive recombinant human protein production by mammary gland of transgenic rabbit necessitates knowledge of the lactation curve and quality (composition) of milk as a possible effect of transgenesis on milk yield.

The use of transgenic animals allows the production of valuable human proteins, enzymes, hormones and growth factors in the milk. By targeting the expression of the transgene product to the secretory cells of animals we may collect and process body fluids with minimal effort. Transgenic rabbit system is a lower cost alternative primarily because rabbits are smaller and thus less expensive to maintain but also because the rabbit reproductive cycle is much shorter than that of the large domesticated animals (**Bozse et al., 2003**).

The mammary gland is the most promising target tissue because it produces large amounts of protein in a temperature-regulated fluid that may be collected daily, in a non-invasive fashion. Production of recombinant proteins in the mammary gland of transgenic animals is dependent on gene promoters used in transgene constructs. Most of the studies have been carried out with the ovine ß-lactoglobulin, bovine α-lactalbumin, caprine ß-casein

or mouse whey acidic protein (WAP) promoter (**Pollock et al., 1999**). The mouse WAP promoter has been used in basic biological studies, as well as for the synthesis of pharmacologically active human proteins, to direct the expression of heterologous genes to alveolar epithelial cells (**Van Cott et al., 2001, Chrenek et al., 2002, 2005, 2007a,b,c**).

The lactation specificity of the regulatory regions used in control of mammary gene expression in transgenic animals is very important in case of expression of foreign biologically active proteins, because these proteins may exhibit their biological functions in the animal if secreted prior to the tight junction formation in the mammary epithelial cells (**Chen et al., 2002**). The effects of expressing growth factors, hormones, oncogenes, ECM components and receptors on mammary gland development and differentiation have been well documented (**Jhappan.et al., 1993**). These effects range in phenotype from impaired lubulo-alveolar development (**Jappan.et al., 1993**), to decreased milk protein synthesis during lactation, without effects on glandular epithelium in activated Ha-ras mice (**Andres et al., 1988**), constitutive milk protein gene transcription in c-myc mice (**Andres et al., 1988**), reduction in milk protein synthesis and lactation deficiency in transgenic mice (**Nemir et al., 2000**), to premature dedifferentiation of secretory epithelial cells and a decrease in milk protein mRNA levels in beta1-integrin mice (**Faraldo et al., 2002**), to hyperplasia and delayed involution in human FGF4 transgenic mice (**Morini et al., 2000**).

Rabbit milk quality varies depending on various factors, as a breed, nutrition, lactation stage or number of pups (**Chrastinova et al., 1997, Lukafahr et al., 1983**). To investigate the effect of over-expression of hFVIII on milk quality of transgenic females, basic analysis of milk was performed and compared with non-transgenic milk at the same conditions. Although our results of transgenic rabbit milk samples have showed that all transgenic females analysed in this work produced lower or higher concentration of rhFVIII with confirmed biological activity (**Chrenek et al., 2005, 2007a,b,c**), significant differences were found only in the content of milk fat and lactose. The higher variability of rhFVIII concentration may be explained by different copies of integrated gene, site of transgene insertion or its genomic environment, what could influence its expression (**Salvo-Garrido et al., 2004**). Relatively higher protein content in transgenic rabbit milk samples may be explained by the production of recombinant human factor VIII. Insignificant difference between transgenic and non-transgenic protein content is in agreement with the previous report where it was showed, that expression of recombinant protein into mammary gland of transgenic animals did not automatically result in any increase in total milk protein content (**Wilde et al., 1992**). Significant differences obtained in our milk samples are in agreement with the range of

variability, where an average of fat content is 12-15g/100g, protein is 8-12.5g/100g, lactose 1.0-2.0g/100g and ash is 2-3% (**Jenness, 1982**). Significantly higher number of somatic cells in transgenic rabbit milk comparing to non-transgenic ones may be explained by used technique of milk collection and temperament of female. Some females during milk collection "cooperated" better than the other did. It is necessary to note also, that milk was collected using "home made air vacuum pump" what may cause a higher number of somatic cells in transgenic milk samples.

Palmer et al. (2003) recently reported that transgenic mice expressing recombinant human protein into mammary gland, under mouse WAP promoter, exhibit defects in lactation and impaired mammary gland development. The WAP promoter was shown to be less efficient than the β-lactoglobulin promoter at driving the over-expression of recombinant human protein into milk (**Barash et al., 1999**). On the other hand, **Van Cott et al. (2001**) concluded that transgenesis and recombinant human protein secretion in milk was not connected with any abnormality concerning milk production such as mastitis or other mammary gland disorders of transgenic pig. Mammary gland specific over-expression of IGF-I did not also impact lactation performance in swine (**Monaco et al., 2005**).

Histological analysis of mammary gland

Our recent histological analyses of lactating transgenic rabbit mammary gland tissues have observed no changes, when compared to non-transgenic mammary gland tissues (**Dragin et al., 2006**). Ultrastructural measurements of cellular organels showed no differences in cellular structure of mammary tissue, but significant differences in relative volume of mitochondria and vacuoles between transgenic and non-transgenic ultrastructure of mammary gland epithelium were observed (**Dragin et al., 2006, Chrenek et al., 2009**).

The presence of the mWAP-hFVIII transgene in rabbit genome and secretion of rhFVIII into milk of transgenic females did not have any adverse phenotypic effect on the offspring and milk quality. All transgenic rabbit females of F_1, F_2, F_3, F4, F5 generation had normal reproductive behaviour, producing litters ranging from 7 to 9 offspring, which is in accordance with our previous results (**Chrenek et al., 2005, 2007a,b,c**).

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