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The Applications of Transgenic Rabbits in Agriculture and Biomedicine

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Abstract: During the last decades transgenic rabbits have provided suitable biological model for regulating and manipulating of interest genes. Several studies showed transgenic rabbits can be produced by different methods. In the last two decades pronucleotide microinjection was conventional and common method for produce transgenic rabbits but the current studies demonstrated that SMGT method provides a simple and straightforward technology to introduce DNA into rabbits which has many advantages in comparison with other methods. The rabbit as both a laboratory and domestic animal species provides several opportunities for investigators to study the mechanisms of human disease such as lipoprotein metabolism, atherosclerosis and hypertrophic cardiomyopathy. Regarding to the present review transgenic rabbits in the last decade could have been managed to provide an alternative way to produce therapeutic proteins for treating human diseases.

Key words: Transgenic rabbit, bioreactor, human disease, microinjection, SMGT, therapeutic protein

INTRODUCTION

Transgenic animals production used as a common technique in the laboratories to study gene function in development and disease to device new animal models of human genetic diseases or to produce recombinant protein in fluids, mostly milk and biomedical science of transgenic animals (Giraldo *et al*., 2000). Transgensis was first described in 1981 (Gordon and Ruddle, 1981) by DNA microinjection of micro ova. The first transgenic rabbits were produced using pronuclear microinjection (Pursel *et al*., 1985). Rabbits as both a laboratory and domestic animal species in genetically and physiologically close to humans so transgenic rabbits are suitable as animal models for various human disease both genetic and acquired. Transgenic rabbits are used as bioreactors to produce pharmaceutical protein and they are an excellent models for the study of lipoprotein metabolism and atherosclerosis (Bosze *et al*., 2003).

History of transgenesis (Foundational technologies): The first chimeric mice were produced during the 1970s. The cells of two different embryos of different strains were combined together at an early stage of development (8 cells) to form a single embryo that in turn developed into a chimeric adult showing characteristics of each strain. Subsequently this was done with other animals as well. Using DNA microinjection of micro ova.

Transgenesis was first in 1981 (Gordon and Ruddle, 1981). Since the development of transgenic super mice in 1982 and the development of first mice to produce a human drug TPA (tissue plasminogen activator to treat blood clots) in 1987 (http;//users.wmin.ac.uk/~reswayk/lecture/transgenic.html). It has biien gaining application among biotechnologists. The term Transgenic refers to an animal that integrates recombinant DNA in its genome and the transferred gene is called a transgene. Inthe early 1980s Several scientific paths converged to establish transgenic animal technologies. During the 1990s, a series of new techniques and modeling systems extended the scope, utility and commercial aspects associated with animal transgenesis even further.

WHY DEVELOP TRANSGENIC RABBITS?

The rabbit (*Oryctolagus cuniculus*) is phylogenetically closer than rodents to primates. The rabbit is also large enough to allow non-lethal monitoring of physiological changes. Because of these reasons some research groups have decided to use the transgenic rabbit as an animal model to study hypertrophic cardiomyopathy, lipoprotein metabolism and atherosclerosis (Bosze *et al*., 2003). The transgenic rabbit system is very crucial because it fills an important gap between the lab mouse and larger domestic mammals. Size wise it is the smallest domesticated animal that can be used to create recombinant protein in its milk both on an

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experimental and a commercial level. The only domesticated species of this animal is the European rabbit but there are >50 established breeds. For the purpose of lab research, uniform stocks of several breeds have been developed including the albino New Zealand White (5-6 kg adult body weight) and the agouti Dutch Belted (~2.5 kg adult body weight). The domestic rabbit is usually ready for mating activity by about 4.5 months of age. In response to mating activity, the rabbit ovulates and cyclic estrous behavior can occur with 5-6 day intervals. The gestation period is about 31 days. A few days before parturition, the pregnant mother builds a nest with her own body hair and straw where available. Transgenic animals are a powerful method to study how genes control the development, growth, maturation and senescence of animals.

The production of transgenic rabbit is very time consuming because rabbits have a longer gestation period than mice and also rather extensive. A number of important parameters that may increase the frequency of transgene integration in mice have been described but there isn't much information about rabbits and other species because there parameters have not been systematically investigated. Thus, the success rate (the rate of integration in pups screened) in the rabbits is lower than that in mice. There are 5 methods for production of transgenic rabbits.

Ponclear microinjection: Pronuclear injection is the most effective and practical method for the generation of transgenic rabbits. DNA construct is injected to the pronuclear of embryonic zygote, using a fine pulled needled operated by a micromanipulation system. The researches have studied to improve the efficiency of transgenic animals production.

Page *et al*. (1995) produced transgenic mice using cytoplasmic injection of DNA mixed with polylysine. The efficiency rate of transgenic animal production was doubled by co-injecting restriction endonucleasetogether with foreign DNA into mouse pronuclei (Seo *et al*., 2000). Pronuclear microinjection is the primary method to produced transgenic rabbits (Massoud *et al*., 1991).

The efficiency of this method for transgenic rabbits production is low ranging from 0.3-2.5% due to factors such as low pregnancy rate, small litter size, cannibalism, mosaicism low transgene transmission rates, low rate of transgene incorporation into the genome of microinjected embryos and stability of transgene transmission to offspring. Hirabayashi *et al*. (2000) visualized pronuclei by zygote centrifugation and produce transgenic rabbits. The effect of DNA concentration on the rate of transgenesis was also tested.

Double pronuclear Microinjection (DM) was the successful technique to produce transgenic mice (Kupriyanov *et al*., 1998) and successfulnuclear transfer in rabbits was first reported by Stice and Robl (1988). There is a significant difference blastocyst survival rate between double (66%) and single (90%) microinjection (Chrenek *et al*., 2005). Nuclear transfer with genetically modified donor cells could eliminate limitation mentioned up (Bosze *et al*., 2003).

In 1983 produced 6 live young mice by modification Robl procedure after transferring 8-16 cell embryonic nuclei into enucleated mature oocytes followed by electric pulse-induced activation and fusion. Collas and Robl (1990) improved McGrath and Solter's technique by using multiple direct-current pulses to induce fusion and activation. Later-stage embryos (32-64 cell stage) with an activated embryonic genome as donors of nuclei in several laboratories have been used successfully (Collas and Robl, 1990; Yang, 1991). Cloned rabbits from somatic nuclei could be gained just from freshly collected cumulus or follicular cells (Chesne *et al*., 2002; Challah-Jacques *et al*., 2003).

Vectores-mediated gene transfer: Gene transfer is medical by means of carrier or vector (a virus or plasmid) for increasing the probability of expression. To transfer genetic material into the cell retrovirus so transmission of the transgene is possible only if the retervirus integrates into the germ cells.

YAC or BAC vectors can be useful for the genes coding for pharmaceutical proteins because they have large upstream regulatory sequences, full length genomic sequences and cDNA sequences which lack intros (Montoliu, 2002). The first transgenic rabbits production using a 250 kb YAC construct containing the wild type mouse tyrosinase gene followed the first mouse YAC transgenics and it could control the hypo pigmented phenotype and retinal abnormalities associated with albinism (Brem *et al*., 1996).

A smaller tyrosinase construct showed great variable ability to overcome pigmentation in Albino hybrid rabbits (Aigner *et al*., 1996). Transgenic rabbits were produced with a YAC vector have high levels of apolipoprotein A (apo A) expression (Rouy *et al*., 1998). Mammalian Artificial Chromosomes (MACs) which have same advantage of artificial chromosome type vectors are replicated and inherited in a stable manner along with other chromosomes in mammalian host cells (Hadlaczky, 2001).

Embryonic stem cell technology nuclear transfer: ES cell technology has been developed significantly in many mammalian species, including hamster mink (Woods *et al*., 2003). Pigs (Zhou *et al*., 2003), sheep (Zhou *et al*., 2003),

cattle (Lee *et al*., 2005) and human (Kubota *et al*., 2000). In mice, genetic manipulation can be applied to ES cells. After genotyping (e.g., knockout), the ES cells are injected to an embryonic blastocyst. If success after breeding, chimeric mice which containing the transgenic genotype germ line cells are born. The gene targeted transgenic animals were produced in mouse just by the ES cell method. Chimeric rabbits were created using some ES cells but germ-line transmission of transgenes from these animals was not reported by development of a cloning technique based on nucleus transmission from a cultured somatic cell into an enucleated oocyte can overcome some limitation. A viable alternative to the ES cell route to product transgenic farm animal were provided by transfected nuclear donor cells screening for transgenesis.

Sperm mediated gene transfer: The first successful cloning of a rabbit from an adult somatic cell was reported by Renard. None obvious morphological abnormalities in the offspring (Chesne *et al*., 2002). Transgenic rabbits expressing Green Fluorescent Protein (GFP) used to identify somatic cells for cloning (Boulanger *et al*., 2002). Rabbit sperm could bring gene to egg at fertilization. Transgenic mice were produced by adding exogenous foreign gene to the medium in which sperm was incubated before *in vitro* Fertilization (IVF).

About 30% of offspring after embryo transfer were transgenic (Lavitrano *et al*., 1989). Successful sperm mediated gene transfer of foreign DNA in rabbit were observed in several years ago (Wang *et al*., 2003; Shen *et al*., 2006). This method (Lavitrano *et al*., 2006) showed that the quality of semen is an important factor in SMGT experiment. In 2003 Wang and his fellow researchers used washed and lipofectin incubated sperm cells and showed that the Bovine Serum Albumin (BSA) incubation medium could effectively block exogenous DNA uptake in rabbit sperm cells.

Thus in the SMGT experiments the concentration of BSA in the extender must be considered. Embroy transfer is used for production of transgenic rabbits. As a medium to transfect testicular germ cells with expogenous DNA via repeated direct injection into animal testis in 2006. Shen *et al*. (2006) employed Dimethylsulfoxide (DMSO). Animal sperm cells of many species could uptake DNA successfully *in vitro* condition (Simth and Spadafora, 2005).

Testis-Mediate Transfer Gene (TMTG): Transgenic rabbit was produced by mating with a male rabbit in which an exogenous gene had been injected in to testis (Shen *et al*., 2006). The benefits of Testis-Mediate Transfer Gene (TMTG) technique are simplicity, high efficiency and low cost in comparison with the conventional microinjection (Simth and Spadafora, 2005; Lavitrano *et al*., 2006). This method requires considerable skill for micromanipulation (Vasicek *et al*., 2007). Rabbit models are not useful just to study human disease mechanistically but also to develop pharmaceutical compounds be tools or method for therapeutics.

CLINICAL APPLICATION

Models of human disease: Models of cardiac structure function relationship and hypertrophic cardiomyopathy. An effective way of finding relationships of cardiac structure-function is transgenesis using cardiac-specific expression. *In vivo* models were not useful to create data referring physiological and pathological states in the heart such as cardiac hypertrophy and dilation. Mouse genome can be generated easily and the cost of maintaining large colonies in comparison with the other animals colonies is low so mice were used for most molecular investigations of the cardiovascular system.

The study of the cardiovascular system is more effective than use of gene-targeted and transgenic mice (James and Robbins, 1997). Crucial facets of human cardiovascular physiology accurately (Kass *et al*., 1998). Fundamental models to duplicate human pathological states using the analogous genetic mutations of human genes expression in the mouse were unsuccessful to create some important parts of human phenotype. There are many differences between murine and human hearts such as component of the cardiac sarcomere in large quantities, the Myosin Heavy Chain (MHC) consists of the fast MHC isoform (a-MHC) in the mouse and the slow MHC (b-MHC) in the healthy human adult which are indicated at the molecular and protein levels (Schiaffino and Reggiani, 1996).

Rabbit is appropriate to move selected models into larger mammalian transgenic animals for appreciation of structure function relationships of the cardiac contractile proteins fully and study a variety of human heart diseases because gestation is short (30 days) and sexual system will be mature approximately rapidly (20-24 weeks). Pursel *et al*., 1985; Weidle *et al*., 1991). Rabbit atria and ventricles express a-MHC and both a and b-MHC isoforms with b-MHC the main adult isoform, respectively at all developmental stages. MHC expression in rabbit is very similar to human heart and it is suitable to evaluate human cardiac function. According to last research data in man man the most current reason of hypertrophic cardiomyopathy is a single point mutation in the β-myosin heavy chain which is diagnosed through hypertrophy in the left ventricular because of absence of an increased

external load. In the transgenic rabbit model to study hypertrophic cardiomyopathy both wild type and the mutant human β-myosin heavy chain cDNA were added to rabbit genome separately under the control of the murine β-myosin heavy chain promoter (Marian *et al*., 1999). In this model, expression of the mutant β-myosin heavy chain is very similar to human patients that are including: earlier death, hypertrophic cardiomyopathy, myocyte disarray, interstitial fibrosis and normal systolic function (Nagueh *et al*., 2000).

Analyzed the myocardial contraction and relaxation profile using tissue doppler imaging (2000) and the effect of Simvastatin on cardiac hypertrophy and interstitial fibrosis (Nagueh *et al*., 2000). In 2001, Nagueh showed that there is a regression between hypertrophy and fibrosisin β-myosin heavy chain mutant transgenic rabbits using Simvastatin treatment. No distinct pattern of hypertrophic cardiomyopathy about different levels of mutant light chain expression was obtained through transgenic rabbits expressing mutant myosin light chain with β-myosin heavy chain promoter as controller (James *et al*., 2000).

To express a reporter gene in transgenic rabbits used murine α and β-cardiac promoter's myosin heavy chain genes (James *et al*., 2000). No apparent harmful impacts have observed under transgenic expression of alpha-MHC and it was as a cardioprotective for *in vivo* tachycardia-induced cardiomyopathy (James *et al*., 2005). One of the cause of familial hypertrophic cardiomyopathy can be mutations in Cardiac troponin I (cTnI).

There are basic differences in Ca^{2+} handling during contraction/relaxation and in alterations in Ca^{2+} flux in the mouse and human heart diseases. Sanbe *et al*. (2005) showed that rabbit can be reflected the human system and they demonstrated expression of modest amounts of cTnI146Gly resulted in serious defects but it doesn't have great effect on cardiac function undesirable. In the pathogenesis of heart failure both enhanced sympathetic drive and altered autonomic control are involved.

The chronically enhanced sympathetic drive was indicated in Transgenic (TG) rabbits with over expressed cardiac Gsalpha which is led to enhanced LV function, heart rate and impaired reflex autonomic control (Nishizawa *et al*., 2007). In human heart diseases, phospholamban is accepted as a pharmaceutic to control decreased calcium (Pattison *et al*., 2008) so transgenic rabbits with high levels of phospholamban expression were died because wasting of severe skeletal muscle. There is significant differences in phenotype between transgenic rabbits and transgenic mice due to TG rabbits show reaction to alterations phospholamban levels rapidly. TG rabbits attacked by cardiac pathology have premature death and phospholamban-overexpresding. Striated muscle fiber was reproduced in the mouse by these murine promoters whereas it wasn't seen in the rabbit.

However, both promoters were able to deliver a high level of transgene expression to the cardiac compartment demonstrating the feasibility of cardiac-selective transgene expression (Bosze *et al*., 2003). Both murine a and b-MHC promoters were able to drive high levels of transgene expression in the rabbit (James *et al*., 2000).

The rabbit as a model for studies of lipid metabolism and atherosclerosis: Rabbits because of several characteristics are a suitable model to investigate lipoprotein metabolism and atherosclerosis. Chemical composition and apoprotein content of apolipoprotein (apo) B-containing lipoproteins in rabbit and human is closed to each others (Chapman, 1980) and apo B mRNA does not process in rabbits liver so apo B-100 with Very Low Density Lipoproteins (VLDL) production is not the same as a human. The cholesteryl ester transfer CETP content is abundant in both of human and rabbit plasma which have an important role in the atherosclerotic process (Duff, 1935; Tall, 1993). Mouse in comparison with rabbit, apoB mRNA and generating apoB 48 containing lipoproteins edited in mouse liver and High Density Lipoprotein (HDL) is the main lipoprotein in the mouse plasma which is resistant to diet-induced atherosclerosis (Paigen *et al*., 1990). Fan *et al*., 1994 produced first transgenic rabbits to study over expression human hepatic lipase (Fan *et al*., 1994).

Overturf and Loose-Mitchell (1992) showed diet with high levels of cholesterol led to severe hypercholesterolemia and develop atherosclerosis rapidly in New Zealand White rabbits (Overturf and Loose-Mitchell, 1992). Inherited abnormalities in lipid metabolism in some rabbit strains cause to produce useful transgenic rabbits. Watanabe in 1980 created Watanabe Heritable Hyperlipidemic Rabbits (WHHL) which are different genetically and followed familial hypercholesterolemia and they were very useful to demonstrate relationship between hypercholesterolemia and atherosclerosis (Watanabe, 1980). Also the St. Thomas rabbit strain was suitable model to study familial combined hyperlipidemia (La Ville *et al*., 1987). Both rabbit strains are usable to cure dyslipoproteinemic patients. To find a quantitative trait locus (QTL for serum HDL cholesterol) backcross progeny of the AX/JU susceptible-hyper responsive strain and the IIIVO/JU hypo responsive strain of rabbit was utilized (Van Haeringen *et al*., 2001). Apolipoprotein A, (Apo A), a glycoprotein which has similar structure to plasminogen and exists just in old world monkeys and

humans naturally. Lipoprotein (a) complex is consist of apo A and apoB-100 that they combined through disulfide linkage. Elevated plasma levels of Lp (a) were correlated with an increased incidence of cardiovascular disease stroke and restenosis (Utermann *et al*., 1989; Maher and Brown, 1995). Relation between human apoA with rabbit endogenous apoB forming Lp (a) complexes was seen using 2 independently created transgenic rabbit lines whereas the same Complex formation construct was not observed in transgenic mice (Rouy *et al*., 1998; Fan *et al*., 2000; Fan and Watanabe, 2000).

Correlation between altered plasma lipid levels and development of atherosclerosis using expressing various apolipoproteins or lipid processing enzymes was studied by many groups. Transgenic rabbit strains such as double transgenic rabbits (Barbagallo *et al*., 1999) or WHHL hich is created by simple cross-breeding (Emmanuel *et al*., 1996; Hoeg *et al*., 1996; Fan *et al*., 2001) make new idea for interactions between protein and lipoprotein metabolism.

Transgenic mice and rabbits were produced by Franz and coworker in 1999 using the 2.3 kb rabbit Smooth Muscle Myosin Heavy Chain (SMHC) as a promoter which is combined with the luciferase gene as a reporter and this promoter is different in vascular and visceral smooth muscle cells in both species (Franz *et al*., 1999). Atherosclerosis is high levels of plasma cholesterol, especially of Low Density Lipoprotein (LDL) cholesterol. Oxidized LDL (oxLDL) is one of the atherogenic agents (Ross, 1993; Glass and Witztum, 2001; Libby, 2002). In ammation is thought to be the link between hyperlipidaemia and atherosclerosis (Ross, 1993; Glass and Witztum, 2001; Libby, 2002). Nissen showed that atherosclerotic coronary lesions regression in humans using treatment of apoA-1.

MMP-12 resulted in media destruction, pseudo aneurysm formation and increase plaque growth quickly in an animal model which is too resemble to atherosclerosis in humans (Tjwa *et al*., 2006). Conjugated linoleic acids, atherosclerosis and hepatic Very Low Density (VLD) lipoprotein metabolism were studied by McLeod *et al*. (2004). One of the major reasons of morbidity and death in developing and industrialized world is Coronary atherosclerosis and its thrombotic complications.

The role of thrombosis on disrupted atherosclerotic plaques is very important in acute coronary syndromes. One of the critical factors in both plaque vulnerability and thrombogenicity is macrophages concentration, these particles are capable to uptake a high amount of 18F-FDG (FDG). There is a correlation between macrophage content within aortic atherosclerosis and PET FDG activity (Zhang *et al*., 2007). Zhang *et al*. (2008), compared obese and lean rabbits for plasma Nonesterified Fatty Acid (NEFA) and Triglyceride (TG) kinetics 2008 also search about and showed lipid metabolism in diet induced obese rabbits is more closely resemble to obese humans (Zhang *et al*., 2008).

The appropriate conditions to indicate lesions were investigated in the rabbit atherosclerosis model and diet with 1% High Cholesterol (HCD) and early start group HCN is the simplest condition to induce rabbit atherosclerosis model (Shimizu *et al*., 2009). The rabbits were used to study atherosclerosis in a large models and one of important model for this study the laboratory rabbit is fed by cholesterol with different methods of lesion induction.

Transgenic rabbits as therapeutic protein bioreactors: One of the most cost-effective ways for the production of valuable recombinant therapeutic protein id the use of live bioreactors for the expression of human genes in the mammary gland of transgenic animals.

The therapeutic proteins production using genetically transformed microorganisms such as bacteria and yeast is extremely an inexpensive and safe technique (Palomares *et al*., 2004). Biological active proteins are associated with inadequate post-translational processes and mammalian cell expression systems were suitable strategies to product these proteins (Simmons and Yansura, 1996; Andersen and Krummen, 2002).

Techniques were used for the stable genetic transformation of cell lines production and mammalian cell culture are expensive and challenging processes (Molowa and Mazanet, 2003). Drugs production with high specific activity is often essential for biosynthetic machinery of eukaryotic cells (Werner *et al*., 1998). Wall (1996) did some research on the process of genetically altered animals which are expressing recombinant proteins in their tissues and secreting them into body fluids. His research helped improve this process (Wall, 1996).

One of suitable and valuable systems which are appropriate to product recombinant proteins involving eukaryotic post-translational modifications is the mammary gland (Clark, 1998). According to an economic point of view transgenic rabbit production is cheaper due to the fact that rabbits are smaller than other domesticated animals also the cost of maintaining them is lower and their reproductive cycle is shorter than other domesticated animals.

However, mice are useful species primarily to test transgene constructs but the main advantages of rabbits in comparison with mice are up to 50 mL of milk production per day (Van den Hout *et al*., 2001), they are easily milked (Duby *et al*., 1993) well traits of their milk

Table 1: Therapeutic recombinant proteins

Express protein	Genes	References
Interleukin 2 (IL29)	Rabbit β-casein-genomic	Buhler *et al.* (1990)
Human growth hormone	Mouse whey acidic protein-genomic DNA	Limonta *et al.* (1995)
Human insulin-like growth factor	Bovine αs1-casein expression casette-cDNS	Brem *et al.* (1996), Wolf *et al.* (1997), Zinovieva *et al.* (1998)
Bovine chymosin	Bovine αs1-casein expression casette *prochymosin* gene	Brem *et al.* (1996)
Erythropoietin	Rabbit whey acidic protein-cDNA	Rodriguez *et al.* (1995)
Erythropoietin	Rabbit whey acidic protein-cDNA and genomic DNA	Massoud *et al.* (1991)
Erythropoietin	Ovine β-lactoglobulin-cDNS (fusion protein with human α-lactoglobulin)	Korhonen *et al.* (1997)
Salmon calcitonin	Bovine αs1-casein expression casette-genomic DNS	McKee *et al.* (1998)
Human nerve growth factor β	Rabbit whey acidic protein-single βα chain	Coulibaly *et al.* (1999)
Equine chorionic gonadotropin	Rabbit whey acidicprotein-single βα chain	Galet *et al.* (2001)
Bovine follicle stimulating hormone	Bovine αs1-casein expression casette-cDNA for α and β chains	Coulibaly *et al.* (2002)
recombinant viral proteins rotaviruses	recombinant VP2 and VP6 proteins	Soler *et al.* (2005, 2007)

(Baranyi *et al.*, 1995), maintaining of rabbits in Specific Pathogem Free (SPF) conditions and not any identified infectious agent pathogenic for humans in the rabbit (Besenfelder *et al.*, 1998). The protein content of rabbit milk is 2.5 and 4.8 times much than sheep and goat, respectively.

A number of therapeutic recombinant proteins have been produce from transgenic rabbit milk or blood as shown in Table 1. In 1997, Lubon and Paleyanda showed transgenic protein with specific γ-carboxylation in some species and Rodriguez and coworkers were observed species-specific glycosylation in 1995 and they reported expression levels of hEPO in transgenic rabbits is lower than transgenic mice with the same construct. Because of different glycosylation pattern, activity of hEPO in rabbit milk in comparison with the activity of natural hEPO is higher (Rodriguez *et al.*, 1995). Aigner *et al.* (1999) implied various splice patterns of RNA growth hormone construct from a human expressed in the mammary glands of transgenic mice, rabbits and pigs.

Monoclonal antibodies, hormones and bioactive peptides and therapeutic proteins which are medical products can be achieved using transgenic rabbits. Scientists could produce monoclonal antibodies through technique of microinjection of constructs containing cloned genes for the light and heavy chains of specific mouse monoclonal antibodies that 2 transgenic rabbit with serum levels of 150-300 μg mL^{-1} were found (Weidle *et al.*, 1991). Weidle *et al.* (1991) showed intact binding sites for the antigen by purification of antibody but only a small fraction of the transgenic antibody was seen which is closely similar to the mouse monoclonal antibody using some isoelectric method. Raju *et al.* (2000) reported species IgG glycosylation.

Rabbit plasmacytoma cell lines production and rabbit-rabbit hybridomas creation to produce rabbit monoclonal antibodies are possible by production of c-myc and v-abl transgenic rabbits (Spieker-Polet *et al.*, 1995). Active Human Insulin-like Growth Factor-1 (hIGF-1) with high-level expression in specific tissue was obtained by over 6 generations in transgenic rabbits (Zinovieva *et al.*, 1998). Ligand blot analysis of the rabbit milk showed relationship between mammary gland specific production of hIGF-1 and of the IGF-1 binding protein IGFB-2 which may have protected from major biological effects within the transgenic animal (Wolf *et al.*, 1997).

The conversion of superoxide anionic radicals is done by superoxide dismutase which enzyme has role in interesting protective reactions (Omar *et al.*, 1992). Human Extracellular Superoxide Dismutase (hEC-SOD) has long half life, expressed highly in whey fraction of transgenic rabbit milk (Stromqvist *et al.*, 1997) and biochemical analysis indicated that both of recombinant hEC-SOD and the native human protein have similar structure of a tetrameric Nglycosilated metalloprotein with an intact C-terminus with a low level of proteolytic activity in rabbit milk. Kazazian *et al.* (1995) reported the cause of Hemophilia A which is inherited bleeding abnormality linked to X chromosome is mutations in the gene for blood clotting factor VIII (FVIII).

By cell culture systems Garber (2000) produced recombinant human factor VIII (rhFVIII) whereas this factor produces commercially now a days (Garber, 2000). Researchers investigated the process of post-translational modifications in the mammary gland in some species such as comparison of hFVIII gene constructs expression in transgenic pigs (Paleyanda *et al.*, 1997), sheep (Niemann *et al.*, 1999) and rabbits (Hiripi *et al.*, 2003) and showed localization of the transgene hFVIII human on chromosome in F2 and F3 generation of transgenic rabbit (Chrenek *et al.*, 2007; Krylov *et al.*, 2008) and indicated this results are the same as reciprocal chromosome painting between the rabbit and human (Krylov *et al.*, 2008). To cure patients who involved hereditary angioedema with C1 inhibitor deficiency (Larrick and Thomas, 2001), a commercial firm (Pharming Group N.V., The Netherlands) tested rabbit milk which is obtained from recombinant human C1 inhibitor. The rabbit mammary gland was used in this firm to produce the recombinant human alpha-glucosidase that this

production is injected as a replacement therapy for Pompe disease (Van den Hout *et al*., 2000). Van den Hout *et al* (2001) demonstrated that the effect of the purified alpha-glucosidase in humans in Phase II clinical trials using transgenic rabbit line expressing 2.4 g L^{-1} was expanded into a colony of several hundred animals. Rotaviruses are the most main reason in infantile viral gastroenteritis. The rate of mortality in this disease is almost 500,000 deaths each year especially in industrialized world (Soler *et al*., 2005, 2007). The first transgenic mammal (rabbit) bioreactors played an important role in co-production of two recombinant viral proteins in milk rapidly as a vaccine (Soler *et al*., 2005, 2007).

Alkaline phosphates are successful pharmaceutics in the gram-negative bacterial Lipopolysaccharide (LPS) which is associated with acute and chronic diseases directly. Purified Tissue Nonspecific Alkaline Phosphatase (TNAP) despite to other alkaline phosphatase isozymes isn't there in large amount from tissue sources could analyze its effect in rabbit transgenic (Bodrogi *et al*., 2006).

Dimethylsulfoxide-sperm-mediated gene transfer technique which is capable to express human lactoferrin protein in the correct tissue was used to produce transgenic rabbits (Bodrogi *et al*., 2006; Li *et al*., 2006). Kondo *et al*. (2008) suggested using transgenic rabbits for retinal degeneration which have large eyes, handling and breeding easily. They created a Transgenic (Tg) rabbit model to study pathophysiology, new strategies to treat retinal degeneration and design the pattern of degeneration through histology and electrophysiology too (Kondo *et al*., 2008).

Rabbits are very good choice, considering both economical and hygienic aspects, for the mammary gland-specific expression of recombinant proteins. Research and pharmanceutical companies are fpcusing their attention on large-scale production of protein using transgenic rabbits (Bosze *et al*., 2003).

CONCLUSION

For modeling and further researching some human diseases and bioreactors can produce trandgenic rabbits using several methods such as pronuclear microinjection (this method is still the major and most popular method for the creation of transgenic rabbits), Retrivirus-mediated gene transfer, Eembryonic stem cell technology, Nuclear transfer, Sperm mediate gene transfer, Tennis Mediate Transfer Gene (TMTG). According to the review, rabbits have short generation interval in respect to large animals, multiple offspring per litter, stable paternal transmission of the transgene and milk yield is more effective for production of a therapeutic recombinant protein in sufficient level with biological activity. For the reason mentioned above, a number of research groups have chosen to use the transgenic rabbit as an animal model to study hypertrophic cardiomyopathy. Lipoprotein metabolism and atherosclerosis and the use of the acquired date for humans.

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