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Review

Transgenic Rabbit Models: Now and the Future

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Abstract: Transgenic rabbits have contributed to the progress of biomedical science as human disease models because of their unique features, such as the lipid metabolism system similar to humans and medium body size that facilitates handling and experimental manipulation. In fact, many useful transgenic rabbits have been generated and used in research fields such as lipid metabolism and atherosclerosis, cardiac failure, immunology, and oncogenesis. However, there have been long-term problems, namely that the transgenic efficiency when using pronuclear microinjection is low compared with transgenic mice and production of knockout rabbits is impossible owing to the lack of embryonic stem cells for gene targeting in rabbits. Despite these limitations, the emergence of novel genome editing technology has changed the production of genetically modified animals including the rabbit. We are finally able to produce both transgenic and knockout rabbit models to analyze gain- and loss-of-functions of specific genes. It is expected that the use of genetically modified rabbits will extend to various research fields. In this review, we describe the unique features of rabbits as laboratory animals, the current status of their development and use, and future perspectives of transgenic rabbit models for human diseases.

Keywords: transgenic rabbits; genetically modified rabbits; rabbit models for human diseases; pronuclear microinjection; genome editing technology

1. Introduction

Genetically modified animals can be very useful tools for biomedical research. They are defined animals whose DNA has been altered using genetic engineering techniques, which include transgenic (carrying exogenous gene), knockout (disrupted endogenous gene by gene targeting), and knock-in (inserted gene sequence at a particular locus by gene targeting). Since the first birth of a transgenic mouse produced by pronuclear microinjection in 1980 [1], numerous genetically modified animals have been generated. In the biomedical research field, the mouse is the most used species for genetic modifications because of well-developed genetic engineering techniques that can produce not only transgenic, but also knockout animals by gene targeting techniques using embryonic stem (ES) cells [2]. In fact, various genetically modified murine models have been produced, which have strongly contributed to the progress of biomedical science. The mouse is one of the most useful laboratory animals, but it may not be omnipotent. Some physiological features of mice are quite different from those of humans, such as lipid metabolism [3–5]. Therefore, the mouse may not be suitable for research of lipid metabolism and atherosclerosis. To extrapolate findings from animal experimentation to

humans, it is essential to choose an appropriate species that conforms to the purpose of the study. Therefore, the development of genetically modified non-murine models has been desired in several research fields where the mouse is considered as unsuitable.

In 1985, the first production of a transgenic rabbit was reported by Hammer et al. [6]. To date, many transgenic rabbits have been generated and used as both human disease models and bioreactors that produce valuable physiologically active substances in their milk [3,4,7–10]. However, in comparison with mice, the transgenic efficiency when using pronuclear microinjection is low and the production of knockout rabbits is difficult because ES cells have not been established for gene targeting in the rabbit [8,11]. These problems limit the use of genetically modified rabbits as human disease models. Therefore, studies to improve the productive efficiency of transgenic rabbits [12–16] and establish rabbit ES cells [17,18] have been conducted for a long time. The emergence of novel genome editing technology such as Zinc finger nuclease (ZNF), transcription activator-like effector nuclease (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), have changed production of genetically modified animals including the rabbit [19–21]. These techniques have revolutionized gene engineering technology because knockout animals can be produced easily with high efficiency by these techniques without ES cells. The first knockout rabbit generated by genome editing technology using ZFN was reported in 2011 [19]. After production of a knockout rabbit by the CRISPR/Cas9 system in 2014 [21], the number of reports of knockout rabbits for human disease models has increased rapidly [20,22–27]. The use of genetically modified rabbits will expand to various purposes in biomedical research, although they have been limited to only transgenic use for a long time.

Transgenic rabbits as human disease models have shown utility in several research fields in which the mouse is unsuitable as a model animal or as a superior model to the mouse, and they fulfill the need for a medium-sized animal between small rodents to humans for translational research. In this review, we introduce the unique features of the rabbit as a laboratory animal, the current status of their development and use, and future perspectives of transgenic rabbit models for human diseases.

2. General Features of the Laboratory Rabbits

The origin of laboratory rabbits is domesticated from wild rabbits (*Oryctolagus cuniculus*) that inhabited the Iberia district of the Mediterranean Sea coast. The domestication of the wild rabbit began around the 11th century in the southern part of Europe and some domestic breeds began to appear from the 15th to 16th century [28]. Then, domestic rabbits, which were developed to various breeds adapted for use as pets and the production of fur and meat, spread worldwide through Europe. The use of rabbits as a laboratory animal started from the mid-19th century and they have been used for studies in physiology, microbiology (infectious diseases), and immunology (vaccines) [29].

At present, laboratory rabbits are generally thought to have unique features as follows: a medium size (i.e., not small like a mouse or rat and not as large as a dog or monkey), ease of handling and breeding in the laboratory because of their tame nature and high reproductivity, blood sampling and intravenous injection are easy because of their large ear, and they are post-coital ovulation animals with a double uterus and large cecum. For safety and development studies of new drugs, rabbits have been used for pharmacokinetics, reproductive and developmental toxicity, local irritation tests, and pyrogenic tests. For research use, the rabbit has been used in the fields of developmental biology and reproductive physiology. As a result of that the rabbit is a post-coital ovulation animal, it is easy to estimate the time of ovulation and developmental stages of embryos. The rabbit was the first mammal to successfully undergo in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) to obtain pups [30,31]. Additionally, the rabbit has been used as an ideal model of atherosclerosis because of its body size, easy manipulation, and extraordinary response to dietary cholesterol [32].

3. History of the Methods to Produce Transgenic Rabbits

3.1. Traditional Pronuclear Microinjection to Generate Transgenic Rabbits

After the first production of transgenic rabbits using the pronuclear microinjection method by Hammer et al. [6], almost all transgenic rabbits have been produced by this method. Pronuclear microinjection remains as the major method to produce transgenic animals, but transgenic efficiency is generally low in rabbits [9,11]. The production efficiency of a transgenic rabbit is thought to be influenced by several factors such as DNA quality (concentration, purity, and size), the skill of microinjection technician, and the colony of rabbit breed. In our laboratory, we have produced more than eight types of transgenic rabbits to study human diseases. The mean efficiency of the transgenic rate was lower than 5% of total born rabbits and 0.5% of the total injected and transplanted embryos [11]. Moreover, using the pronuclear microinjection method, we cannot control the gene copy number or the insertion site in a chromosome. Therefore, we sometimes encountered the "position effect". In this case, we may not be successful in demonstrating protein expression, even though we have successfully generated transgenic founders. The expression levels of the transgene may depend on both the insertion site and copy number. Therefore, several alternative transgenic methods to pronuclear microinjection for improvement of the transgenic efficiency and stability have been explored in rabbits. For example, the sperm vector [12,33,34], ICSI-mediated transgenesis [35,36], transgenesis by somatic cell nuclear transfer (SCNT) [37] or chimeric SCNT [13,38], lentiviral vectors [14], transposon-mediated transgenesis [15,39], and novel genome editing technology [16,40,41] have been examined to produce transgenic rabbits. The characteristics of these major historical techniques are summarized in Table 1. Currently, the most expected alternative candidate that is better than traditional pronuclear microinjection should be novel genome editing technology. In particular, the CRISPR/Cas9 mediated genome editing technique is a powerful tool to generate genetically modified animals. Recently, the detail of genome engineering technologies in rabbits was well reviewed by Song et al. [42].

Table 1. History of transgene methods for the production of transgenic rabbits.

Reported Year/Technique	Remarks	Ref.
1985 Pronuclear microinjection	The gene copy number and insertion site in a chromosome are uncontrollable (the position effect will sometimes occur). Low efficiency (less than 5% of total born rabbits and 0.5% of total injected embryos) [11]	[6]
2006 Sperm vector (SMGT, TMGT and ICSI-mediated)	High efficiency (48.4–85.7% of total born rabbits) Simple and high performance but unstable (authors could not obtain transgenic offspring)	[12,33–36]
2009 SCNT (somatic cell nuclear transfer)	Very difficult technique (efficiency was 1.2–3.5% of reconstituted and transferred embryos)	[13,37,38]
2010 Lentiviral vector (simian immunodeficiency virus)	The founder rabbit will be a mosaic. Transgenic offspring can be obtained if transgenes integrate into sperm or oocytes, but at a low transmission rate. High efficiency in F0 (32% of total born rabbits and 9.4% of total injected embryos)	[14]
2013 Transposon mediated gene transfer (Sleeping beauty)	A single copy of the transgene was integrated and maintained for multiple generations (≥4) without any sign of epigenetic silencing of transgene expression. High efficiency (15.2% of total born rabbits and 1.5% of total injected embryos)	[15,39]
2016 Novel genome editing technology (CRISPR/Cas9 mediated gene transfer)	Knock-in rabbits The knock-in efficiency rate decreases with kb-sized (large) donor DNA. HDR enhancer RS-1 increases knock-in efficiency. High efficiency (26.3–35.0% of total born rabbits and 6.8–7.0% of total injected embryos) Off-target effects remain.	[16,40,41]

SMGT, sperm-mediated gene transfer; TMGT, testis-mediated gene transfer; ICSI, intracytoplasmic sperm injection; SNCT, somatic cell nuclear transfer; CRISPR, clustered regularly interspaced short palindromic repeats.

3.2. Novel Genome Editing Technologies to Generate Knock-In (Transgenic) Rabbits

In the past decade, the emergence of novel genome editing technologies, such as ZNF, TALEN, and CRISPR/Cas9, has dramatically changed the rate of progress in current biomedical science. In 2012, Jinek et al. [43] found that Cas9, which is a component of the CRISPR/Cas system, is a DNA endonuclease dependent on RNA, which generates site-specific double-stranded DNA breaks (DSBs). In 2013, several groups succeeded in genome engineering of mammalian cultured cells using the CRISPR/Cas9 system [44,45]. This technique allows deletion and displacement of a specific DNA sequence with high efficiency and ease. The genome editing by the CRISPR/Cas9 system is much simpler and easier than ZNF and TALEN, and shows high efficiency. It enables the generation of knockout animals using fertilized eggs of any species without established ES cells.

CRISPR was discovered as an acquired immunity system (i.e., an exclusion system for foreign DNA) in *Eubacterium* and *Archaebacterium*. The Cas9 protein forms a complex with gRNA that has a homology sequence of target DNA and induces a site-specific DSB. In the CRISPR/Cas9 system, it is only 20 bases in the gRNA of approximately 100 bases, which prescribe the target sequence of the DSB. Therefore, only 20 bases need to be changed for each target and all other base sequences and Cas9 are common. Additionally, the gRNA can be easily obtained from a manufacturing company. As a result of these reasons, the CRISPR/Cas9 system underwent rapid widespread use, even by inexperienced researchers, in molecular biology experiments as a genome editing tool.

The genome editing by CRISPR/Cas9 has potential benefits to not only generate knockout animals, but also produce knock-in animals by gene integration into specific DSB sites of host DNA when the donor ssDNA (subject genetic sequence including the homology arm of both sides) is added to gRNA and Cas9 mRNA or Cas9 protein at microinjection into embryos. The integration of donor DNA was limited to the small single-stranded oligodeoxynucleotide (ssODN) and the insertion efficiency was low at first, but the integration rate of kb-based ssDNA has increased owing to the improvement of genome editing technology. Recently, Song et al. [16] reported that RS-1, a homologous recombination (HDR) enhancer, increases the efficiency of producing knock-in rabbits through the genome editing technique. They knocked in enhanced green fluorescent protein (EGFP) and tdTomato genes into the Rosa26 locus of the rabbit by this method and reported knock-in efficiencies of 26.3–35.0% of total born rabbits and 6.8–7.0% of the total injected embryos [16,29]. Using this method, the knock-in efficiency should be more than 10-fold that of traditional pronuclear microinjection.

In 2014, Kaneko et al. [46] reported successful introduction of endonuclease mRNAs into zygotes to induce editing at targeted loci and efficiently produced knockout rats by the electroporation method with a high survival rate. It is noteworthy that electroporation of ZFNs resulted in an embryonic survival rate of 91% and a genome editing rate of 73%. This method has some advantages compared with the traditional microinjection technique. Using this method, specialized skills for microinjection are not needed and a large number of zygotes can be manipulated at one time. The electroporation technique may accelerate the production of genetically modified animals because of its simplicity and ease. New technologies and techniques applicable to the production of genetically modified animals are developing and improving daily.

4. Transgenic Rabbits for Human Disease Models

Transgenic rabbits as human disease models [47–115] are listed in Table 2.

Table 2. Transgenic rabbits for human disease models.

Possible Disease Models/Transgene	Remarks	Ref.
Lipid metabolism and atherosclerosis		
Human apo(a)		[47]
Human apo(a) and apoB		[48]
Human apo A-I		[49,50]
Human apoA-I/C-III/A-IV gene cluster		[51]
Human apoA-II		[52]
Human apoB		[53]
Human apoB-100		[54]
Human APOBEC1		[55]
Rabbit apobec-shRNA	RNA-i knockdown of rabbit APOBEC1	[56]
Human apoC-III	Knockout was also produced [27]	[57]
Human apoE2	Knockout was also produced [25,26]	[58]
Human apoE3		[59,60]
Human CETP	Knockout was also produced [22]	[61]
Human CRP		[62]
Human EL		[63,64]
Human HL		[65,66]
Human LCAT		[67,68]
15-Lipoxygenase type 1	Osteoporosis and periodontal disease [69,70]	[71]
Human LPL	Obesity [72]	[73,74]
Rabbit MMP-1		[75]
Human MMP-9		[76]
Human MMP-12	Inflammation [77]	[78]
Human PLTP		[79]
Human Urotensin II		[80]
Cardiac failure		
Hypertrophic cardiomyopathy/cardiac protein		
Rabbit α-MyHC		[81–84]
Rabbit cThI-G146 mutation		[85]
Rabbit Cardiac Gsα		[86,87]
Rabbit ELC1v-M149V mutation		[88]
Rabbit Phospholamban		[89]
Human β-MyHC-R403Q mutation		[90]
Rabbit β-MyHC-R403 mutation		[91,92]
RyR2 R4497C		[93]
Proarrhythmia		
Human KvLQT1-Y315S mutation (LQT1)	Long QT syndrome	[94,95]
Human HERG-G628S mutation (LQT2)		[94,95]
Human KCNE1-G52R mutation (LQT5)		[96,97]
Immunology		
Human HLA-A2.1	Immunity to viral infection	[98]
Rabbit FcRn	Humoral immune response	[99]
Rabbit IgH	B cell-deficient (B cell development)	[100]
Human CD4	AIDS	[101–103]
Human CD55 and CD59	Xenotransplantation	[104]
Tumor (oncogenesis)		
Rabbit EJ-ras DNA	Papilloma, keratoacanthomas and squamous cell carcinoma	[105,106]
Rabbit c-myc oncogene	Lymphocytic leukemia	[107]
Rabbit E-κ-myc oncogene	Lymphoid tumor	[108]
Other models		
Bovine GH	Acromegaly and diabetes mellitus	[109]
EGFP	Marker for tissue engineering and regenerative medicine	[110]
Oct4 promoter-EGFP	Marker to investigate rabbit embryo development	[111]
Ovine PrP	Rabbits are susceptible to a various prion isolates [116].	[112]
Rabbit Rhodopsin-P347L mutation	Retinitis pigmentosa	[113]
Human VEGF	Hepatic hemangioma (Kasabach-Merritt syndrome), and renal dysfunction	[114,115]

APOBEC1, APOB mRNA editing protein; CETP, cholesteryl ester transfer protein; CRP, C-reactive protein; EL, endothelial Lipase; HL, hepatic lipase; LCAT, lecithin: cholesterol acyltransferase; LPL, LPL, lipoprotein lipase; MMP, matrix metalloproteinase; PLTP, plasma phospholipid transfer protein; MyHC, myosin heavy chain; cTnI, cardiac troponin I; ELC1v, essential myosin light chain 1; RyR2, cardiac ryanodine receptor. HLA, human leukocyte antigens; FcRn, neonatal Fc receptor; GH, growth hormone; EGFP, enhanced green fluorescent protein; PrP, prion related protein; VEGF, vascular endothelial growth factor.

The most-used research areas of transgenic rabbits as human disease models are lipid metabolism and atherosclerosis. In fact, thus far, more than 20 genes related to lipid metabolism and atherosclerosis have been introduced into transgenic rabbits (Table 2). Transgenic rabbits have also been used as disease models for cardiac failure (hypertrophic cardiomyopathy and proarrhythmic), immunology (immunity to viral infection, humoral immune response, and transplantation), and oncogenesis because of the physiological features of laboratory rabbits. The most important physiological feature of rabbits is that they have a similar lipid metabolic system to humans, whereas the mouse is quite different (Table 3) [3–5]. In mice, the major lipoproteins in plasma are high density lipoprotein (HDL) owing to the lack of cholesterol ester transfer protein. Furthermore, because rabbits do not have hepatic apolipoprotein-(apo)B mRNA editing activity, rabbit apoB-48 is only present in intestinally derived chylomicrons, which is similar to humans, unlike mice and rats in which apoB-48 is contained in both chylomicrons and hepatically derived very low density lipoprotein and low density lipoprotein (LDL). There are other differences between humans, rabbits, and mice, such as the localized patterns of hepatic lipase (HL), an important enzyme in the metabolism of chylomicron remnants, and HDL. In humans and rabbits, HL is bound to a proteoglycan on the surface of hepatic or endothelial cells, whereas 70% or more HL in mice is in its free state and is released into blood without binding to the cell surface. Therefore, mice are resistant to cholesterol-rich diets and have difficulty in developing hypercholesterolemia and atherosclerosis. The characteristic details of transgenic rabbit models for lipid metabolism and atherosclerosis have been described in several reviews [3,7,8]. Interestingly, among these reviews, Fan and Watanabe [3] mentioned that phenotypes are different between mice and rabbits even when the same genes are introduced. For example, the expression of either human HL or lecithin cholesterol acyltransferase (LCAT) induces enhanced lesion formation in transgenic mice but protects against atherosclerosis in rabbits. Overexpression of apoE in mice inhibits atherosclerosis but leads to increased plasma LDL and spontaneous atherosclerosis in transgenic rabbits. High expression of human lipoprotein lipase results in myopathy in transgenic mice but not in transgenic rabbits. These observations strongly suggest that the difference in the physiological background among species affects the result of studies using human disease animal models. To extrapolate results from animal experimentation to humans, we must focus on the appropriate species depending on the purpose of the study. In addition to lipid metabolism, the myocardium composition of rabbits is also similar to that of humans (Table 3). In the human heart, β-myosin heavy chain (β-MyHC) comprises 90% of total myofibrillar myosin. In the rabbit heart, similarly to humans, 80% consists of β-MyCH in contrast to the mouse heart predominated by the other myosin isoform, α-myosin heavy chain (α-MyCH), at 95% [9,117]. These physiological features of the rabbit can be useful for human disease models of cardiac failure, such as hypertrophic cardiopathy and proarrhythmia. To date, novel transgenic rabbits have been continuously produced and used as human disease models in lipid metabolism and atherosclerosis. Unique transgenic rabbit models have also been reported in other research areas such as Rhodopsin P347L transgenic rabbits for retinitis pigmentosa [113] and Ovine PrP transgenic rabbit for prion disease [112,116] (Table 2). In the future, it is expected that the use of transgenic rabbits will expand to various research fields except for lipid metabolism, atherosclerosis, and cardiac failure.

Table 3. Comparison of lipid metabolism and cardiac characteristics between mice, rabbits, and humans.

	Mouse	Rabbit	Human
Lipoprotein profile	HDL-rich	LDL-rich	LDL-rich
CETP	None	Abundant	Abundant
Hepatic apoB mRNA editing	Yes	No	No
apoB48	VLDLs/LDLs and chylomicrons	Chylomicrons	Chylomicrons
Hepatic lipase	High, 70% in circulation	Low, liver-bound	Low, liver-bound
Hepatic LDL receptor	Usually high	Down-regulated	Down-regulated
apoA-II	Monomer	Absent	Dimmer
Dietary cholesterol	Resistant	Sensitive	Sensitive
Atherosclerosis	Resistant	Susceptible	-
Myosin type of myocardium	α-MyHC	β-MyHC	β-MyHC
Ion channel of myocardium	I_{to} and I_{kslow}	I_{kr} and I_{ks}	I_{kr} and I_{ks}
ECG pattern	J-wave (Single lead)	T-wave (12 lead)	T-wave (12 lead)

HDL, high density lipoprotein; LDL, low density lipoprotein; CETP, cholesteryl ester transfer protein; VLDL, very low density lipoprotein; MyHC, myosin heavy chain; ECG, electrocardiogram. Modified from Fan et al. [3,5].

5. Transgenic Rabbit as a Bioreactor

Lastly, we will briefly discuss transgenic rabbits as bioreactors. In a bioreactor, recombinant proteins are expressed in mammary gland epithelial cells and produced in the milk of transgenic animals. By purifying the recombinant proteins from the milk, useful substances can be obtained at lower costs than conventional production methods such as cell culture systems [10,118]. For this purpose, large animals are believed to be suitable as bioreactors because they produce a large amount of milk. In contrast, rabbits require a simple means of farming compared with larger livestock. Breeding rabbits in a laboratory is also possible and they mature early (5–6 months of age) [119]. Their reproductive capacity is high and they deliver six to eight pups per birth. Additionally, rabbits do not have seasonal reproductive cycles and the amount of milk per lactation period is 1–1.5 L [119]. Therefore, rabbits are quite suitable for small scale production and allow rapid development of recombinant proteins. Many researchers have reported the production of valuable physiologically active substances using transgenic rabbits [3,7,8].

The first therapeutic protein produced by a bioreactor for a human disease was antithrombin III (Atrin®) from goat milk, which was approved in 2006 in the EU and 2010 the USA. Therapeutic protein from rabbit milk have also been approved, such as C1-esterase inhibitor (Ruconest®) for hereditary angioedema in 2010 (EU) and 2014 (USA), and Factor VIIa (Sevenfact®) for hemophilia A or B and inhibitors in 2020 (USA).

6. Conclusions and Future Perspectives

The development of gene engineering technology has enabled the generation of transgenic rabbits that have contributed to the progress of biomedical science as an animal model in several research fields where the mouse is unsuitable as a research model. To extrapolate results from animal experimentation to humans, we must focus on the appropriate species depending on the purpose of the study. Rabbits have some unique features such as a lipid metabolism system very close to humans and a medium body size (not too small like mouse and rat, and not too large like dog and monkey) that is easily handled and applied to experimental manipulation. Therefore, many transgenic rabbits have been produced as animal models for lipid metabolism and atherosclerosis, cardiac failure, immunology, and oncogenesis. For a long time, the transgenic efficiency of rabbits was low and production of knockout rabbits was difficult because of the lack of rabbit ES cells. However, these problems were solved by the emergence of novel genome editing technology in this decade. We can now easily produce both transgenic and knockout rabbit models to analyze gain or loss of functions, or both. In the future, it is

expected that the use of genetically modified rabbits will expand to various research fields including lipid metabolism, atherosclerosis, and cardiac failure.

Genome editing technology may replace the traditional pronuclear microinjection method to produce transgenic rabbits because of the high efficiency of the knock-in (transgenic) rate. In knock-in rabbits produced by genome editing technology, one transgene is usually integrated at the DSB site in contrast to the pronuclear microinjection method that ranges from two to 100 copies or more. The transgene copy number should be considered when analyzing a target gene if it requires a high expression transgenic model. However, expression of a transgene may be out of proportion to the integrated transgene copy number. Moreover, extremely high expression beyond the physiological tolerance level may cause undesirable effects on animals and will not reflect the condition of the human disease. Additionally, complicated gene manipulation, such as conditional gene knockout in an organ-specific and time-controlled manner using the Cre/LoxP system, may be difficult in rabbits. As a result of that a long time is needed for a change in generation owing to the half year required for sexual maturation of rabbits, and there are no available inbred strains (a closed colony is common), brother–sister mating has difficulty to produce the homozygote genotype needed for the experiment in a short period. Moreover, a relatively large breeding colony is needed to avoid the inbreeding depression caused by brother–sister mating. Thus far, there are no reports about a conditional gene targeting model in the rabbit and it will be necessary to perform further studies.

Lastly, the preservation of useful strains of genetically modified rabbits will become an important task after their production. Currently, the cryopreservation of sperm and embryos has become a common a method to preserve laboratory animal strains, especially mice and rats. By cryopreservation of sperm and embryos, we will be able to reduce costs and space in animal breeding facilities and avoid accidents that might occur when transporting living animals. To maintain rabbit strains, the cost and occupying space in an animal facility will be problems with the increasing number of genetically modified rabbit strains for preservation. The advantages of cryopreservation of sperm and embryos are very useful for rabbits. For rabbits, several studies have reported various procedures for cryopreservation of sperm [120–122] and embryos [123–125], but a standard has not been established yet. In the future, it is expected that the development and establishment of cryopreservation methods for the rabbit and a rabbit bioresource center as a sperm and embryo bank of useful rabbit strains to provide them to researchers worldwide will increase the use of valuable genetically modified rabbits.

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