Rabbit Biotechnology

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Editors

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Rabbit Genomics, Transgenesis, Cloning and Models

Springer

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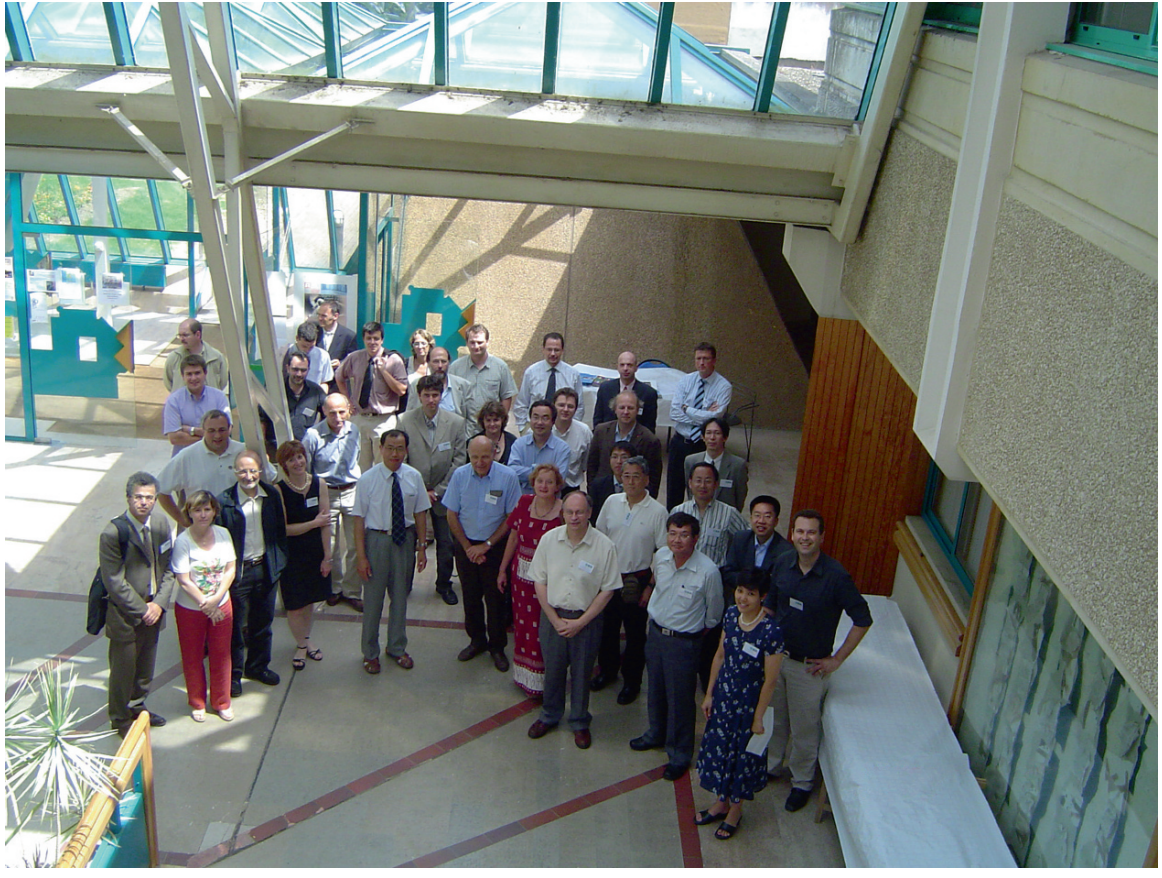
The first international transgenic rabbit conference cut held in Tsukuba, Japan (May 2005)



The 2nd international conference on rabbit biotechnology held in Jouy en Josas, France (June 2007)

Chapter 1
Introduction

Louis-Marie Houdebine and Jianglin Fan

The study of biological functions of proteins and their possible roles in the pathogenesis of human diseases requires more and more relevant animal models. Although mice including genetically modified mice offer many possibilities, other non-murine species are absolutely required in some circumstances. Rabbit is one of these species, which has been widely used in biomedical studies. This animal is genetically and physiologically closer to humans including cardiovascular system and metabolism characteristics. Rabbit is thus more appropriate than mice to study some diseases such as atherosclerosis and lipid metabolism. Because of its larger size, surgery manipulation, bleeding, and turn-over studies are much easier performed in rabbits than in mice. Furthermore, transgenic rabbits can be produced using microinjection and other methods such as lentiviral vectors. Cloning in rabbits has been proved possible, even though still laborious and time-consuming. Hopefully, functional rabbit ES cell lines will be available in the coming years. Gene deletion or knock-out in rabbits will then become possible. In the mean time, gene knock down using siRNA or micro RNA is an attractive alternative. The accomplishment of the whole rabbit genome sequencing is about to be achieved. Moreover, rabbit is being used to produce pharmaceutical proteins, including human polyclonal antibodies. Rabbit is also a significant source of meat in some countries. These biotechnology projects, although very different, are using essentially similar technical approaches. An optimal application of rabbits requires improvement of these different techniques. To exchange the information and update the advanced technology in rabbits, the first international meeting on rabbit

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biotechnology was held in Tsukuba, Japan, in 2005 and followed by the second meeting in Jouy en Josas (near Paris) on June 14 and 15, 2007. The next meeting has been scheduled on June 4 and 5, 2009 in Xi'an, China. A specific biotechnology of the rabbit is therefore emerging. We strongly feel that the time has come to compile a contemporary professional book (tentatively named as Rabbit Biotechnology). This book aims at confronting the different sophisticated approaches developed independently in different academic and industrial laboratories as well as in some companies in the world.

Chapter 2
Improvement of Rabbit Production

Shuji Kitajima

Abstract In this chapter, I will introduce some of the techniques for efficient colony management and production of rabbits. The artificial insemination (AI) can be a useful technique which shows a better performance rather than natural mating. The pregnancy rate and mean litter size after AI are not different from those found after natural mating. Moreover, judging from our result, one ejaculate from one male rabbit can be sufficient to fertilize about 25 female rabbits with AI. In addition to AI, sperm freezing is also an important technique for a stable maintenance of rabbit strains for long time at low cost. These techniques can contribute to an enhancement of productivity and stability of maintenance of rabbit colonies.

Keywords Artificial insemination, Cryopreservation, Rabbit breeding, Sperm freezing

2.1 Introduction

Inbred rabbits are not commonly used, therefore, an appropriate colony management is certainly necessary for the maintenance of rabbit breeds to avoid inbreeding deleterious effects. Colony management implies a lot of rabbits and a large space for breeding and maintenance. However, it is difficult for most laboratories to find enough space for rabbit breeding and maintenance because of the relatively large scale of animal facilities and for financial reasons. Moreover it is necessary to save space and money in many cases.

Currently, in domestic animals, especially in cattle, artificial insemination (AI) using frozen sperm is widely implemented in the world. It is generally considered that this

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technique contributes very significantly to an enhancement of productivity. In rabbits, many studies on AI have been reported. This method is very simple and applicable for systematic colony management rather than the natural mating. There are lots advantages in AI for rabbit reproduction. For instance, we can use diluted semen for AI, usually semen from one male is sufficient to fertilize more than ten female rabbits at one time. It enables rapid expansion of rabbit colony from even small number of rabbits at a start. In the research field, we sometimes need a lot of rabbits in same age at one time for experiments. By reproduction with AI, we can prepare them easily at a given time. Moreover, recent advances of reproductive science have brought us a number of advantages such as cryopreservation of sperm and embryos in liquid nitrogen, which allows us to maintain stably them for long time at low cost. The combination of AI and cryopreservation of rabbit semen can be useful techniques for the maintenance of rabbit breeds. For efficient production of rabbits, AI is a useful technique, it can contribute to an enhancement of productivity and reduction of the number of rabbits for colony maintenance. In this chapter, I introduce a technique of AI and cryopreservation of rabbit semen which are used routinely in our laboratory.

2.2 Artificial Insemination (AI)

2.2.1 Rabbit Semen Collection

Semen collection can be performed using artificial vagina (AV) (Morrell 1995). We can purchase AV from company, but we can made it easily (Morrell 1995; Naughton et al. 2003). The basic structure of the AV is assembled by the appropriate size of outer case and inner rubber which lines over the top and bottom edges of inner case. The test tube is connected to the top hole of the case, and the space between inner case and lined rubber is filled with hot water (Fig. 2.1). The most important point is to control temperature of hot water (It is better within 40–45°C at time of use) placed in the AV since rabbit ejaculates by heat stimulation. If temperature of hot water is too hot, it often induces contamination of urine into ejaculate. A decoy animal used for semen collection is enough to be a male, it is not necessary to be female. When two male rabbits are placed in same box (enough size for adult two rabbits) or decoy male rabbit is introduced in a cage of candidate rabbit for

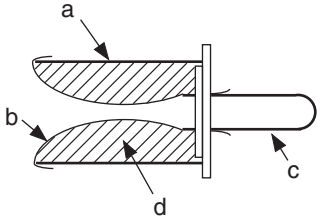

Fig. 2.1 Basic structure of artificial vagina (AV). The AV is assembled by the appropriate size of outer case (**a**) and inner rubber (**b**) which lines over the top and bottom edges of inner case. The test tube (**c**) is connected to the top hole of the case, and the space between inner case and lined rubber is filled with hot water (**d**)

semen collection, well trained rabbit will give mounting action in a few minutes. When male rabbit does the action, lead its penis into the AV immediately to collect the ejaculate (Fig. 2.2). After semen collection, volume of ejaculate should be measured. Sperm concentration and motility should also be counted immediately under microscope. The semen showing contamination by urine, extremely low sperm

Fig. 2.2 Collection of rabbit semen. *Top*: When two male rabbits are placed in same box, well trained rabbit will give mounting action in a few minutes. *Middle*: When male rabbit does the action, lead the penis into the AV immediately to collect the ejaculate. *Bottom*: Photograph shows the collected semen by our handmade AV

concentration, a large proportion of abnormal sperm or a low motility should not be used for AI. It is generally considered that semen collection from a given rabbit should better not be performed more than twice in a week. In our laboratory, mean semen volume, sperm concentration and motility in over 5 months old Japanese White (JW) rabbits (from 5 to 24 months old, n = 202) was 0.53 ml, 600.4×10^6 spermatozoa/ml and 82.7%, respectively.

2.2.2 Insemination

When AI is performed immediately after semen collection, dilution of semen can be appropriate with saline. If there are several hours after semen collection until it is used for AI, semen should be diluted in an appropriate buffer such as tris citrate glucose (TCG) buffer and kept under appropriate temperature (El-Gaafary 1994; Roca et al. 2000). The sperm can maintain a normal fertility for several hours and up to a few days if it is stored in appropriate buffer and temperature (Lopez-Gatius et al. 2005; Roca et al. 2000). (Please see Section 2.5.)

For insemination, the pipettes with a 5 mm diameter of polyethylene, plastic and glass tubes can be used and it needs to be at least 10–15 cm in length. Vagina of rabbits is long and there is distance to the orifice of uterus. Therefore, the pipette should be inserted 10–15 cm into vagina to ensure appropriate delivery of sperm to female rabbits (Fig. 2.3). The glass tube bent slightly at the portion from tip 4–5 cm is easily inserted into the depth of vagina. When insemination is done by two persons, one retains rabbit by holding her back and the other should operate the glass tube. If rabbit is anesthetized by such as thiamylal sodium, which is an ultra-short acting anesthetic, it can be performed safely and surely by one person without any accident. At the time of insemination, ovulation of rabbits must have been induced by injection of 50 U of human chorionic gonadotropin (hCG).

A study on the relationship between the number of spermatozoa for AI and the pregnancy rate has been done by Viudes de Castro and Vicente (1997) who reported that the reproductive performance after inseminating 4 or 10×10^6 total sperm was not different as judged by fertility (74%) and litter size at birth (9.0 pups). On the contrary, female rabbits inseminated with 2 or 1×10^6 sperm had a significantly lower pregnancy rate: 66% and 23%, respectively (Viudes-De-Castro and Vicente 1997). Castellini and Lattaioli (1999) also reported similar results showing that the reproductive performance is independent of sperm number within a certain range. The low sperm motility in samples containing less than 4×10^6, and particularly less than 2×10^6 have a negative effect on the reproduction rate. In our data, the number of 10×10^6 spermatozoa/0.5 ml/doe was mostly efficient in JW rabbit, the pregnancy rate and mean litter size were 80% and 7.6 pups, respectively. These data are not different from those found after natural mating. It can even be considered

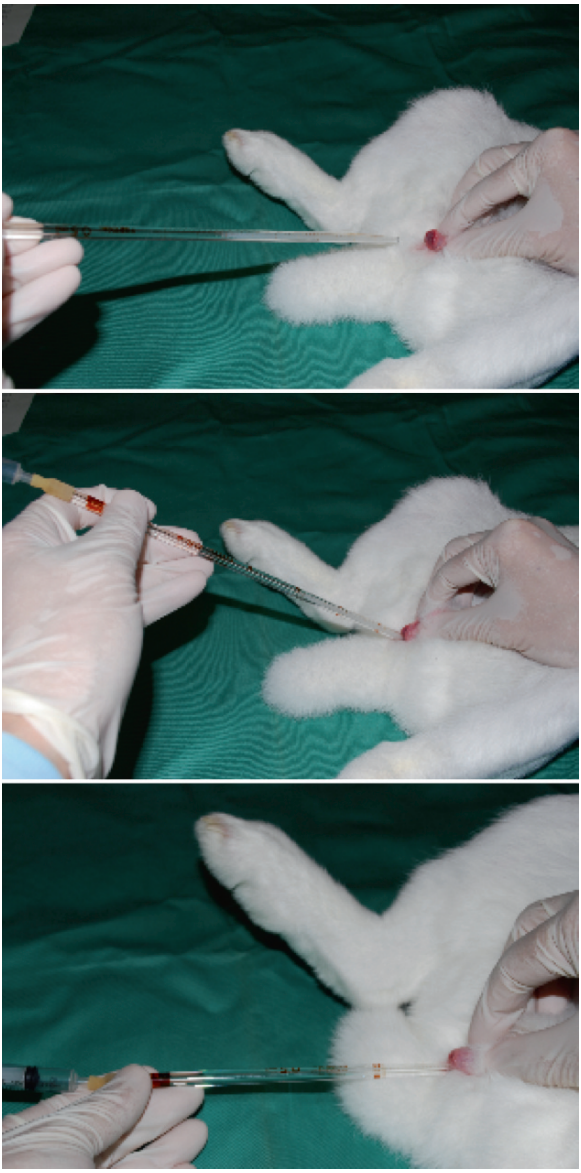

Fig. 2.3 Artificial insemination (AI). *Top*: In the photograph, rabbit is anesthetized by thiamylal sodium which is an ultra-short acting anesthetic. When insemination is done by two persons, one retains rabbit by holding her back and the other should operate the glass tube. *Middle*: The glass tube connected with 1 ml-syringe is inserted into the vagina. *Low*: The pipette should be inserted 10–15 cm into the vagina to ensure the delivery of sperm to female rabbits, because the vagina of rabbit is long and there is distance to orifice of uterus. At the time of insemination, ovulation of the rabbits must be induced by injection of 50 U of hCG

that a better performance is generally observed with AI than with natural mating. Judging from our result in JW rabbits, such as mean value of sperm volume, sperm concentration and motility, one ejaculate form one male rabbit can be sufficient to fertilize about 25 female rabbits using AI.