BREEDING OF MOUSE-DEER FOR AN EXPERIMENTAL ANIMAL AND ITS PHYSIOLOGICAL CHARACTERISTICS

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ABSTRACT

Lesser mouse-deer, *Tragulus javanicus*, was bred for the purpose to develop a new experimental animals in the field of animal science and veterinary medicine. Captive mouse-deer were able to be tame well by hand feeding, although they are nervous and shy in temper. Stainless steel cage (60 cm X 60cm X 60cm) was enough for their breeding. Morphologically, the rumen, reticulum, and abomasum of mouse-deer were similar with those of domestic ruminants, except that the omasum was vestigial. The colon showed a characteristic spiral loop of double strands. The erythrocytes of mouse-deer were markedly small in size and their shape was variety, such as spherical, oval, triangular, disc, and biconcave disc. Unique holes were recognized on the cell membrane of many erythrocytes. They seemed not to perforate the cell, but to be pits of various depth. The membrane of the pits was trilaminar structure as well as other part of erythrocytes. The mouse-deer was able to breed young in the laboratory. It is considered that lesser mouse-deer is very interesting animal to use for research not only as a small ruminant, but also as a ancestral artiodactyls to study the evolution of ruminants and erythrocytes.

Key words : Mouse-deer, Rumen, Ruminant, Erythrocyte

INTRODUCTION

Lesser mouse-deer, inhabits Southeast Asia, is the smallest ruminant in the world (Novak, 1991). Even in adult, the are under 2 kg in body weight (Medway, 1977). In phylogeny they are considered to be the primitive artiodactyls (NRC, 1991). On the viewpoint of the smallest animal among ruminants, we are interested in the mouse-deer to develop a new experimental animal substituting for large domestic ruminants such as cattle and sheep. In this paper we describe the general features and breeding of mouse-deer in the laboratory, and their physiological characteristics examined.

MATERIALS AND METHODS

Lesser mouse-deer were captured from the forest of Selangor and Pahang, Malaysia. They were raised in the stainless steel cage, which were originally produced for laboratory rabbits (60 cm X 60cm X 60cm). The animals were maintained at the institute of Medical Research (IMR) in Kuala Lumpur, Malaysia, and the Universiti Putra Malaysia (UPM). Some animals from each colony were transferred to Japan in 1989 and 1995. Since 1989 we have kept mouse-deer in the National Institute of Animal Health, Tsukuba, Japan (Fig. 1).

For morphological study, dead animals and stillborn young were dissected. The alimentary tracts were fixed in 10 % neutralized formalin solution. Various parts from the tract were processed into 5 μm paraffin sections by routine procedure, and stained with hematoxylin-eosin and Azan for light microscopic examination. Blood samples were collected from healthy animals through saphenous vein of hind limbs. The blood was fixed in cold fixative, 1 %

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Figure 1. A pair of mouse-deer in a stainless steel cage (front: female, rear: male). The male has tusk-like canine at upper jaw and developed chin gland.

glutaraldehyde in 0.1 M phosphate buffer, for electron microscopic examination. After rinsing, a part of the fixed blood cell suspension in phosphate buffer was placed on the glass slips coated with poly-L-lysine and post-fixed in 1 % osmium tetroxide in 0.1 M phosphate buffer. The specimen on the glass slip was freeze-dried, coated with gold, and observed by scanning electron microscopy (JEOL JSM-6301). Remaining fixed blood cell precipitation was resuspended in rat blood plasma, then coagulated by addition of thrombin. The coagulation of blood cells was post-fixed in 1 % osmium tetroxide and embedded in epoxy resin. The ultrathin sections were stained with uranyl acetate and lead citrate, and observed by transmission electron microscope (JEOL 100CX).

RESULTS AND DISCUSSION

Housing

The animal house for the mouse-deer was conditioned to a temperature of 20-25 °C and a humidity of 50-60 %. For the mouse-deer

Figure 2. Complex stomach of mouse-deer. Rumen consist of dorsal sac (Ds), ventral sac (Vs), and blind sac (Bs). Re: Reticulum, Ab: abomasum, Ce: cecum.

breeding, stainless steel cages, which were made originally for laboratory rabbits and connected side-by-side with sliding partition, were used. The sliding partition was used when animals moved from one side to the other at bedding change, and when animals were forced to be separated at fighting. At times, for example nursing infant, the animals used wide space without a partition. Bedding in the cage was commercial wood shavings for the laboratoryrodents, and it was changed once a week. In this housing condition the mouse-deer bred young well.

In the case of mouse-deer breeding at the wide room or floor, they could not settle down. The mouse-deer are solitary animal in nature, and they like enclosed space such as a narrow box or channel to hide. A wide room or space was not suitable for mouse-deer breeding. In the case of wide room or floor breeding, we had to prepare a shelter for each animal to hide.

Lightening was also important for effective breeding. In the animal house lightening was controlled 12h light (6.00-18.00) and 12h dark (18.00-6.00). Bright lightening is not adequate for them. In the field they are most active at dawn and dusk. Even in laboratory, under dim lightening or semidarkness they were calm and moved actively to get food.

Feeding

The mouse-deer were fed on rabbit pellet and crashed cubed hay with additional food, such as banana, carrot, long beans, and cabbage. The food was given once a day at evening (14.00). Pellets and cubed hay were given enough for animal eating *ad libitum*. Drinking water was given in a pan, not by water bottle for rabbits and rats. They drank water using tongue like as dog do.

When food was given, mouse-deer eat banana, carrot, and long beans first, then pellets and cubed hay. To establish the mouse-deer as a experimental animals, we planned to cut down the variety of food, especially additional food like long beans, carrot, cabbage, and banana, because of constant food supply through year. Several years later, we changed banana to sweet potato. For nursing infant, we gave the mixture of mashed banana and canned milk for pig. Not only infant but also mother liked this mixture, and resulted in good reproduction, especially in case of shortage of mother milk.

Slice carrot, sweet potato and banana were good food for hand feeding to tame the animal. The hand-feeding made the animal tame readily, so that we could touch their bodies.

Abrupt change of food should be prohibited. As ruminants keep flora in the rumen, the change of food caused serious damage for digestive function and finally the death of animals might occur. Even if the animal survived, their reproduction was suppressed markedly.

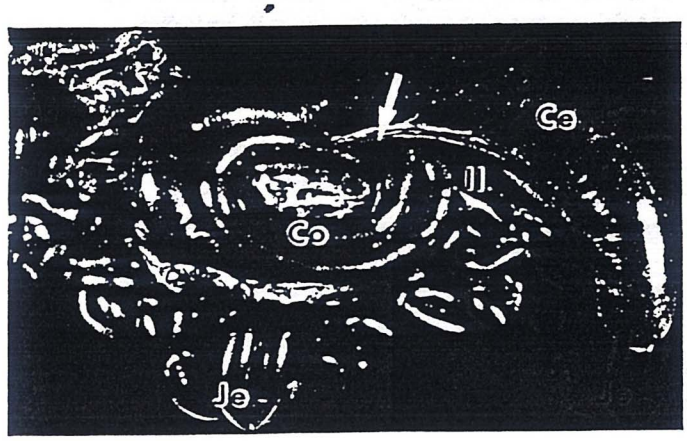

Figure 3. Intestine of mouse-deer. An arrow indicates the connecting site of ilium (Il) to cecum (Ce). Colon (Co) shows characteristic spiral loop. Je: jejunum.

Behavior

In cages, the mouse-deer were rest mostly in day time. They were active at dawn and dusk. The mouse-deer are considered to be nocturnal animal, but they were not so active in the mid-night compared at dawn and dusk. The animals are told to be nervous and shy, but they were accustomed to caretakers. And they did not surprise even if the caretakers made loud noise at the time of bedding change. However, strange person came into the animal house, mouse-deer noticed them, and they were irritated and panicked occasionally. They seemed to be very sensitive on the scent of stranger.

Mating

As mouse-deer were solidarity animals in wild, it is the most troublesome to mate with a partner for reproduction in a cage. Estrous term of female was difficult to identify by watching. In no association with their sex they fought each other and suffered. When the mouse-deer were panicked, they were jumping and dashed to dash to the wall suicidally. Occasionally some animals died at that time. Mating with young partner caused less troublesome compared with matured animals, although the reproduction was delayed.

Morphology and Alimentary Tract

The mouse-deer have a large stomach with 3 compartment, such as rumen, reticulum, and abomasum (Fig.2). Omasum is very small and vestigial. In the infant we could not identify the omasum, but in adult a small area between reticulum and abomasum was confirmed. The reticulo-rumen volume was larger than that of domestic ruminants. And in the rumen there were cellulolytic bacteria and characteristic protozoa, *Isotricha jalaludinii* (Kudo *et al.*, 1997). Therefore, the mouse-deer were considered to become a good model to study ruminology taking place of cattle and sheep.

The cecum was relatively large compare with that of domestic ruminants. The colon made a characteristic spiral loop consisting of one and half-centripetal gyri and two centrifugal gyri (Fig. 3).

Ultrastructure of Red Blood Cells

The most interesting characteristics of the mouse-deer is the smallest erythrocytes among the mammals (Altman and Dittmer, 1961). Erythrocytes of mammals are generally flat disc in shape, usually they are called biconcave disc. However, the erythrocytes of the mouse deer were very small in size and variety in shape, such as spherical, oval, and rod as well as some biconcave disc. In scanning electron microscopic observation, we found a small hole on the membrane of erythrocyte (Fukuta *et al.*, 1996). It was about 135 mm in diameter (Fig. 4). Although the holes were deep and their bottom were hardly observed,

Figure 4. Scanning electron micrograph of erythrocytes. Shape of them are variety as spherical, oval, rod, and disc. Some erythrocytes have a small pit. X 7,000. Scale: 1 μm.

Figure 5. Transmission electron micrograph of erythrocytes with unique pits. The membrane of the pits is trilaminar structure as well as other part of cell membrane. X 20,000.

they seemed not to perforate the cell, but to be pits of various depth. The blood cells were not infected with the parasites by the inspection of Giemsa stained blood smear. And also no hemolysis was recognized in these animals. evagination of the cell membrane, and they were no abnormalities in the cytoplasm and membrane of the cell morphologically. The membrane of the pits consisted of the trilaminar as well as other part of the cell. No sign of the abnormality of the cells were not found by transmission electron microscopy.

The spherical erythrocytes were not adequate for adaptation to change of osmotic pressure. Indeed lower osmosis broke erythrocytes easily in this animal compared with erythrocytes of concave disc in other mammals. The reason of the erythrocyte of the small size and variable of the shape was not clear. Small size of the erythrocyte was compensated by numerous numbers of the cells. Therefore, hematocrit value of the mouse-deer was 50.2 % and almost same level of other mammals (Fukuta *et al.*, 1996).

These unique pits were not observed in other mammals. Some genetic defected animals, which fail band 3 protein of cell membrane, showed holes of the erythrocyte. In these animals severed hemolysis was occurred. The mouse-deer were healthy and no hemolysis occurred. Neither light microscopic or electron microscopic examination revealed parasites and abnormality in cell membrane and cytoplasm (Fig. 5).

In mammals, erythrocyte excludes nucleus at their maturing process, unlike birds, reptiles and amphibians, which contain in the nucleus in matured erythrocyte. The mouse-deer were considered to be primitive mammals, so that the erythrocyte might remain the process of the exclusion of the nucleus. Further studies of the homatopoietic organs were needed to clear the significance of characteristic erythrocytes of the mouse-deer.

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