Artificial Insemination in Common Marmosets using Sperm Collected by Penile Vibratory Stimulation

Authors: Ishibashi, Hidetoshi, and Motohashi, Hideyuki H.

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ARTIFICIAL INSEMINATION IN COMMON MARMOSETS USING SPERM COLLECTED BY PENILE VIBRATORY STIMULATION

Hidetoshi Ishibashi
Hideyuki H. Motohashi

Introduction

Many New World primate species are endangered in the wild by habitat destruction and hunting. Captive breeding programs are needed urgently to help rescue such species, as an adjunct to habitat conservation. Unfortunately, primates may not breed well in captivity, and assisted breeding techniques, such as artificial insemination (AI), are needed to increase the number of individuals and facilitate their effective genetic management. AI offers the potential to exchange genetic material between colonies without the risk of disease transmission or injury inherent in moving animals. Although AI has been used in domestic animals for many years, attempts to transfer this technique to primates have met with limited success (reviewed in Wolf, 2009).

Among members of the Callitrichidae, the common marmoset (*Callithrix jacchus*) has been an important model

species in reproductive research (Hearn, 1986; Dukelow, 1993). Several approaches for marmoset sperm collection have been reported, as outlined below. Epididymal sperm can be collected from surgically dissected epididymis (Morrell *et al.*, 1997). This approach is invasive and not repeatable. It is, therefore, a last resort when other methods cannot be used. Rectal probe electro-ejaculation has been extensively used in larger Old World species (Schaffer *et al.*, 1989), but not widely used in marmosets since it is invasive and therefore requires anesthesia that may depress the neural responsiveness. The third approach, vaginal washing after natural copulation (Morrell *et al.*, 1998), requires intensive observation and suffers from contamination with mucus from the female genital tract. The forth approach, penile vibratory stimulation (PVS) has been described as a repeatable noninvasive method of sampling enhanced quality of semen (Schneiders *et al.*, 2004). However marmoset offspring has not been produced by AI with sperm collected by PVS, and it is this approach we follow in this study.

Methods

Animals

We used common marmoset monkeys as subjects. Animal experiments were approved by the ethics committee for primate research of the National Center of Neurology and Psychiatry, Japan, and conducted in accordance with the institutional guidelines. The marmosets were caged indoors, with light on from 0700 to 1900 hours, temperature at 26 to 28 degree Celsius and humidity at 40 to 60%. The marmosets were fed monkey chow (CMS-1M, Clea Japan Inc.) at 50 gr per day, with a vitamin supplement, and fruit. Water was available ad libitum. Blood samples (0.1 mL) were taken from the femoral vein and plasma progesterone concentrations were determined using an enzyme immunoassay (AIA-360, Tosoh Corp.). The day of ovulation (Day 0) was taken as the day preceding that in which the progesterone concentration had risen from basal levels to higher than 10 ng/mL (Harlow *et al.*, 1983).

Artificial insemination

We artificially inseminated two female marmosets, once for each animal, on Day 0. On the day of artificial insemination, we collected semen by vibratory stimulation of the penis as described previously (Kuederling *et al.*, 2000) with minor modification as follows. Unsedated male marmoset was placed on a holding apparatus (CL-4532, Clea Japan Inc.) and its stand in a dimly-lighted room. Prior to and after semen collection, animals were given an edible reward. As a vibrator, we used an electric toothbrush with a frequency of 117 Hz (DB-3, Minimum Corp.) or 100 Hz (Clinica, Lion Corporation), fitted with a 5.5 or 6.5 mm o.d. silicone tube. The ejaculated semen was collected into a tube containing 200 microliter of sperm washing medium (1012, SAGE In-Vitro Fertilization, Inc.) in the first case, or a tube containing 50 microliter of test yolk buffer (90128, IS Japan Co., Ltd.) in the second case, and was suspended by gentle pipetting. The sperm was checked for motility and was purified in the first case: the suspension was centrifuged at 500 g for 5 minutes and pellet was subjected to swim-up purification. A female was sedated with an intramuscular administration of the mixture of 70 microgram of midazolam and 14 microgram of butorphanol tartrate per kilogram of body weight and anesthesia was maintained by inhalation of isoflurane or sevoflurane. The animal was placed, dorsally recumbent, on the holding apparatus with the pelvis slightly raised. An 8 cm long sterile catheter (NM-AIH10, Nipro) was attached to a 1 mL syringe, inserted into the vagina and the sperm suspension injected.

Results

We have performed artificial insemination twice and obtained normal delivery in both cases. A lineage of marmosets described in this study is shown in Fig. 1. A male marmoset Nukky was subjected to PVS and collected semen was purified and used for AI to a female marmoset Sunny, who conceived and delivered twin babies within the normal gestation period (Day 143). The offspring, Johnny and Jenny, were morphologically normal, raised by their mother, and developed normally (Fig. 2). To examine the sexual capacity of AI offspring, Johnny was subjected to PVS after the sexual maturation. Collected semen was used for AI. The inseminated female, Ayataka, conceived and delivered twin babies within the normal gestation period (Day 148). The offspring, Suzume and Tsubame, were morphologically normal, raised by their mother, and developed normally (Fig. 2).

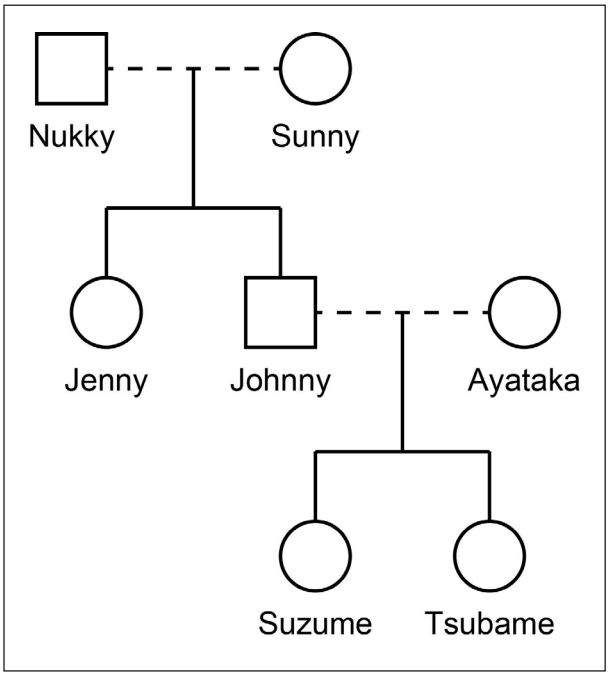

Figure 1. Lineage of marmosets used in this study. Square: male. Circle: female. Dotted line: artificial insemination.

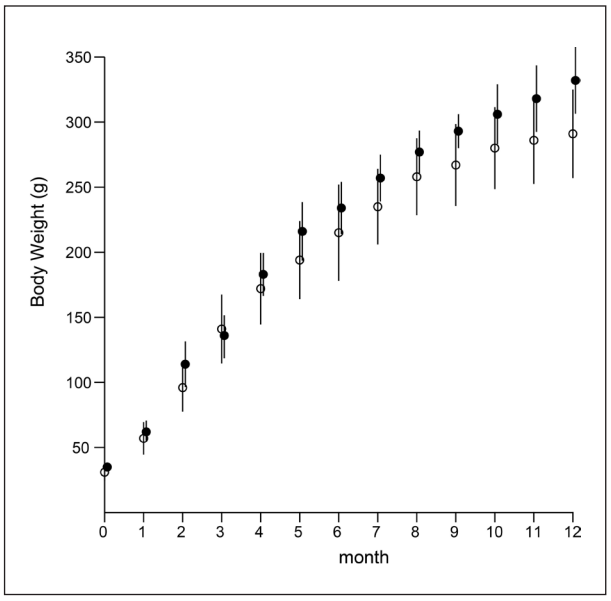

Figure 2. Body weight of offspring born after artificial insemination. Open symbols: average of the institute. Filled symbols: average of AI offspring. Error bar denotes standard deviation.

A female marmoset Jenny, born by AI, has been a subject of embryo collection study, and offers normal embryos repeatedly (results will be described elsewhere). Other two offspring, Suzume and Tsubame, have not reached sexual maturity as of manuscript preparation and thus their fertility has not been confirmed.

Discussion

In this study we have shown, for the first time, the production of primate offspring by AI with sperm collected by PVS. Since conceptions occurred, the viability and fertilizing capacity of the sperm were not adversely affected by the preparation procedure. AI offspring developed normally, and have sexual capacity. Therefore we conclude that AI can be successfully performed with PVS sperm. It is, at present, not clear whether this technique is applicable to wide range of primate species, since seminal fluidity varies among primate species. It is correlated with the mating system of the species: coagulation rating is high in those genera where females mate with multiple partners and low in genera where females are monogamous (Dixson and Anderson, 2002). Chimpanzee and macaque monkeys, characterized by multimale-multifemale social system, show high coagulation ratings. Meanwhile, monogamy is the modal social grouping of any callitrichid taxon (Anzenberger and Falk, 2012). Thus, although the physical characteristics of the other members of the family Callitrichidae are not well-known, it is expected to be rather common among family members. This technique is simple and all instruments except hormone measurement apparatus are easily available. Semen collection by PVS required only one male unlike vaginal washing which requires a pair of animals. Although the subjects in this study are captive common marmosets, the simple features of the technique, i.e., simple procedure, relatively affordable apparatus, and least number of animals, are likely to apply to the breeding of endangered animals belonging to the family Callitrichidae.

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Hidetoshi Ishibashi1 and **Hideyuki H. Motohashi1**, Department of Neurophysiology, National Institute of Neuroscience, NCNP, Japan, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan. E--mail: <ishiba@ncnp.go.jp>