A simplified manual for breeding, husbandry, and management of common marmosets

Miki MIWA*, Naho KONOIKE, Narumi KATSUYAMAS, Katsuki NAKAMURA*

Cognitive Neuroscience Section

Center for the Evolutional Origins of Human Behavior, Kyoto University

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*Corresponding authors

Miki MIWA

Cognitive Neuroscience Section, Center for the Evolutionary Origins of Human Behavior, Kyoto University. 41-2 Kanrin, Inuyama, Aichi 484-8506, Japan.

Katsuki NAKAMURA

Cognitive Neuroscience Section, Center for the Evolutionary Origins of Human Behavior, Kyoto University. 41-2 Kanrin, Inuyama, Aichi 484-8506, Japan.

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Introduction

In recent decades, many experiments have been conducted using the common marmoset (*Callithrix jacchus*) in medical and life sciences, including those in Japan ('t Hart et al., 2012; Kishi et al., 2014). The common marmoset is a promising laboratory animal because of its remarkably high reproductive capacity among primates. However, various challenges exist with keeping common marmosets in Japan, as husbandry methods for this animal remain unclear. Some facilities may also have begun to maintain common marmosets. Here, we compiled a simplified manual on how to breed and raise well-built common marmosets in a captive environment based on our experience at Kyoto University. In a European research facility, animals weighing 400–500 g were used for the experiments. In Japan, animals weighing approximately 300 g are commonly used. This difference in body weight may affect various aspects of this research. In this manual, animals weighing 350 g or more are defined as "well-built marmosets." We focused on many of the descriptions related to breeding, such as the selection of breeding animals, mating methods, and management of pregnancy and perinatal periods. Information on common marmosets is available on websites currently (Common Marmoset Care; EAZA, 2017). Numerous guidelines exist for experiments (EU Commission Recommendations, 2007; National Research Council, 2011). In Japan, a new guideline, "Guidelines for Experiments on Primate Subjects in Neuroscience," has been recently established via the Japanese Neuroscience Society (Japan Neuroscience Society, 2021). We recommend referring to these websites and guidelines for detailed information on marmoset care and experimentation.

[1] Breeding

1. Selection of breeding animals

The selection of breeding animals is crucial for raising well-built common marmosets. Possible selection criteria include age, health conditions, and rearing history.

(1) Breeding age

Common marmosets are adults aged 24 months (2 years) (Abbott et al., 2003). Therefore, the recommended age for breeding in captivity is 18 months (Box & Hubrecht, 1987; Tanioka, 1996; Tardif et al., 2003). If a female becomes pregnant at a very young age, the development of the mother and offspring is stunted (Poole et al.,1999). Common marmoset females can reproduce at older ages because they ovulate until near the end of their lives (Tardif & Ross, 2019); however, the number of litters is significantly reduced from 12 to 15 years of age (Smucny et al., 2004), which also affects maternal milk production (Tardif et al., 2008). Male testosterone levels begin to decline at approximately 7.5 years of age (Tardif et al., 2008). Therefore, the recommended breeding age for captivity is between 2 and 10 years.

(2) Indicators of health conditions

Healthy animals must be selected for breeding purposes. Body weight is the first indicator of health. The average weight of captive common marmosets is approximately 350 ± 50 g for adults (mean ± standard deviation; Araujo et al., 2000). The weight of the breeding female (mother) profoundly affects the number of ovulations and litters, along with milk quality, and is closely related to offspring

development (Tardif & Jaquish, 1997; Tardif et al., 2001). Individuals weighing > 400 g have longer life expectancies and tend to remain healthy into old age (Tardif et al., 2011). The data from our colony showed a significant positive correlation between the weight of the mothers and that of their offspring at 2 years of age (coefficient: 0.67, p = 0.008; Figure 1). In contrast, no significant correlation was observed between the weight of breeding males (fathers) and their offspring (coefficient: -0.05, p = 0.851; Figure 1). Based on these results, to obtain large animals, it is essential to select mothers who are as large as possible, weighing 350 g or more (preferably approximately 400 g), with good muscle, subcutaneous fat, coat, feces/urine, and activity. Mating partners should be as distant family lineage as possible (Tardif & Ross, 2019), preferably four or more relatives. Ensuring the absence of congenital or acquired malformations is crucial. Penile injury or loss owing to the undescended testis and capsule strangulation may be seen in males, while papillary defects, narrow vulva owing to labia minora fusion (Isachenko et al., 2002), and nonporous vagina without a vaginal opening (Niimi et al., 2015) may be seen in females, although they are rare. In males, a long, undamaged penis with a large testis within the scrotum should be selected, while in females, a securely open vaginal opening should be chosen (Tanioka, 1996).

(3) Rearing history

Common marmosets actively participate in child-rearing, not only by the mother but also by the father and siblings. Specifically, they carry newborns on their backs and handle the excrement. The experience of alloparenting, the animals' participation in their younger siblings' parenting during their developmental period, influences their reproductive success (Tardif et al., 1984). Therefore, animals reared by their family of origin until at least 13 months of age and have cared for younger siblings should be selected for

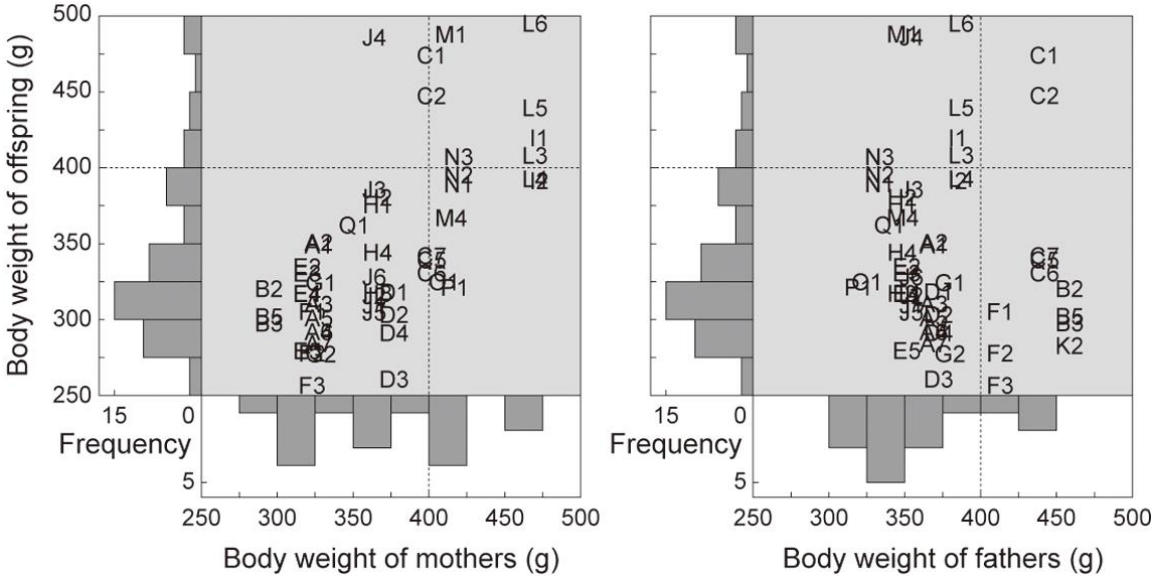

Figure 1 Body weight of offspring at 2 years and that of parents in the common marmoset colony at the Cognitive Neuroscience Section, Center for the Evolutionary Origins of Human Behavior (EHUB), Kyoto University. Data are obtained from 17 parent-offspring pairs, with alphabetic characters indicating the name of the parents and numbers indicating the number of deliveries. Offspring weight shows the mean litter weights (1–3 offspring). A significant positive correlation was observed between mother and offspring weight; however, not between father and offspring weight.

breeding (EU Commission recommendations, 2007; EAZA, 2017). Those animals who actively participated in offspring rearing should be opted for breeding.

2. Pairing

Compatibility is crucial for selecting suitable animals. Pairing is based on compatibility, namely, whether they will accept each other as breeding partners. The first stage involves only visual contact (visualization), without physical contact. Phase 2 involves partial contact in adjacent spaces, while phase 3 involves full contact in the same space. The below descriptions include the details of each phase, the proceeding process, and indicators from male and female responses.

(1) Phase 1: Visual contact

Adjacent male and female spaces are placed so that they can see each other and observe their reactions. The space between them should be separated by a transparent partition such as acrylic or by a distance of 16 cm, in the case of a net, to avoid contact, even if they reach out. Difficulty in adjacency owing to space or cage shape prompts moving animals to a carrying cage (small cage for transportation), which are then placed adjoining each other (Figure 2-1). Alternatively, the carrying cage can be moved closer to the rearing cage (Figure 2-2). Typically, as females are more selective about their mates, placing them in a larger captive cage makes compatibility assessments easier.

(2) Phase 2: Partial contact

If the reaction is positive, physical contact is initiated. Physical contact is encouraged by changing the partition between the two cages from acrylic to mesh, placing the cages close together, or placing the carrying cage with one individual in the other's cage (Figure 2-3). Complete cohabitation is avoided at this

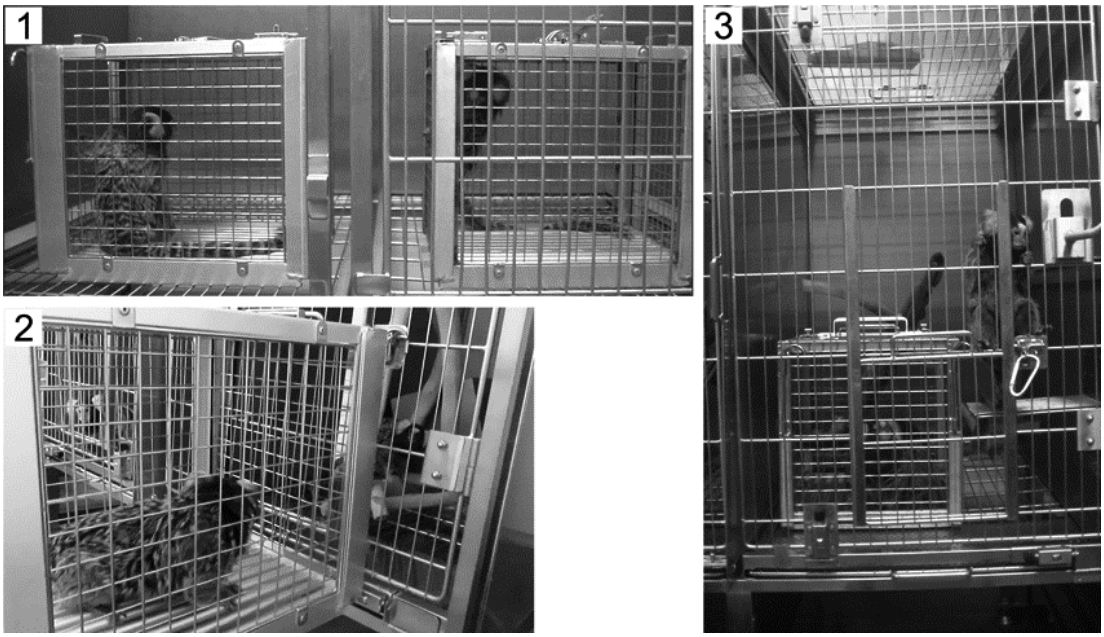

Figure 2 Common marmoset pairing. 1 and 2: visual contact. When the carrying cage is in contact (1) and when placed outside the rearing cage (2). 3: Partial contact. A case of the carrying cage is placed inside the rearing cage.

stage, maintaining the interaction as partial. Immediate separation of animals occurs on an exhibition of aggressive calls (chatter) or behaviors.

(3) Phase 3: Full contact

A positive response to partial contact allows both animals to cohabit in the same space. It is essential to prepare for potential aggression at this stage as there are no physical barriers between them, enabling immediate intervention if needed.

During pairing, the indicators for assessing affinity include courtship behavior (tongue in-out or tongue flickering, rhythmic opening and closing of the upper and lower lips while protruding and retracting the tongue); sexual arousal (male: erection, female: immobilization); and affinity responses (proximity). If these responses are observed, the pairing is considered favorable and proceeds. Conversely, if aggressive or stressful responses are observed, pairing should be suspended, terminated, postponed, or the partner changed. In all phases, the observation time should be 5–15 min per session. When proceeding cautiously, each stage may be conducted once a day for two or three days to a week; however, if the response is positive, the third stage may be conducted in one day. The duration of pairing should be determined based on husbandry conditions and individual responses. During ovulation, males tend to be more responsive, and females are more likely to accept mating, making pairing increasingly potent. Common marmosets do not show external changes such as menses or sexual skin swelling. Thus, it is difficult to determine the reproductive cycle without measuring progesterone and its metabolites in the blood and urine, except in multiparous animals. However, artificially adjusting the reproductive cycle, similar to birth control, as described below, can help identify the ovulation period.

3. Pregnancy and perinatal care

(1) Pregnancy diagnosis and monitoring

The duration between pairing and first childbirth has been reported to be 206 days (median 168.5 days, range 143–441 days) in American colonies and 204 days (range 143–528 days) in British colonies (Tardif et al., 2003). Considering the common marmoset pregnancy period (average 143 days, Tardif & Ross, 2019), it appears that pregnancies often occur approximately 2 months after pairing, with some variation. If pregnancy is unobserved for an extended period, it is advisable to investigate its potential causes. Uterine palpation helps confirm pregnancy and its monitoring (Mitchell & Jones, 1975; Phillips & Grist, 1975) and ultrasound (echographic) examinations (Jaquish et al., 1995; Oerke et al., 1995). Pregnancy confirmation and weight measurements are recommended once a month.

Uterine palpation: It is conducted without anesthesia to induce the animal to move from the housing cage to the carrying cage. Subsequently, one hand is used to grasp the lower back and abdomen, the thumb and index finger are slid down to the armpit while maintaining the grip, and the animal is secured in a natural standing position. The first and second fingers of the other hand are placed on the lower abdomen over the abdominal wall to probe the uterus and measure its diameter (Figure 3). Changes in pregnancy progression are shown in Table 1. Pregnancy progression data were obtained from 20 breeding females by

monthly weight measurements. The estimated gestation days were calculated assuming that ovulation occurred 10 days after corpus luteum regression (owing to delivery or abortion). In nonpregnant cases, a movable, solid, and small spherical uterus can be palpated approximately 0.5–1 cm in depth from the inguinal region. At approximately 1.5–2 months of pregnancy, a uterus with a thickness of over 1 cm, shaped like a square caramel or barrel, can be palpated through the abdominal wall, confirming pregnancy. The number of fetuses in pregnancy can be confirmed after 110 days of gestation when the fetus's head becomes palpable. Initially, the fetal head is positioned in the upper abdomen. Afterward, it gradually descends to the pelvic cavity as the pregnancy progresses. Delivery often occurs approximately one month after the fetal head is palpable in the pelvic cavity.

Figure 3 Palpation of the uterus on the common marmoset. The common marmoset is held in the upright position with one hand, and the diameter is measured by pinching the uterus over the abdominal wall with the first and second fingers of the opposite hand.

Ultrasound examination: Ultrasound examinations were conducted without anesthesia. Typically, two individuals, one performing the examination and the other restraining the marmoset, are needed. Therefore, additional assistance may be beneficial. Similar to the palpation, the lower abdomen is shaved using an

Table 1 Uterine palpation findings

Palpation results	Estimated days of pregnancy (mean ± SD)	N	Features
Non-pregnant	-	-	Uterus diameter: approximately 5 mm Spindle or cudgel-shape
＜P10	40.6 ± 10.0	13	Uterus diameter: approximately 8 mm Small, hard, spherical
P10	57.1 ± 9.1	40	Uterus diameter: approximately 10 mm Barrel shape, caramel shape Rigid and resilient rectangular
P20	80.3 ± 10.8	34	Uterus diameter: approximately 20 mm Barrel shape or caramel-shape Increased elasticity from P10
P30	94.8 ± 9.6	19	Uterus diameter: approximately 30mm Difficult to grasp, overall-shape Soft
Head of litter	120.9 ± 9.8	53	Started palpation of the hard fetal head. The number of litter can be checked

electric shaver after capturing and restraining the individual. In unshaved cases, olive oil can be used instead of ultrasound gel to obtain distinct images. First, the restrainer holds the animal using a thumb and forefinger under the animal's armpits while the other fingers gently grab the animal's abdomen. The animal is maintained in a sitting position while securing pelvic movement with the opposite thumb and forefinger. The animal is held in a slightly backward-tilted seated position, making the examination easier. Providing the preferred food during the procedure can help keep the animal calm. Another assistant, when present, can dispense food; otherwise, the restrainer can provide food while the examiner holds the lower body. In non-pregnant cases, the transverse image of the uterus is oval. The non-pregnant endometrium is tightly closed and appears as a single white line through the center of the transverse image. The uterine lumen is not visible (Figure 4-1). When the embryo is implanted into the endometrium, a split endometrium is observed as two white lines, and the enclosed cavity becomes visible. Separation of the endometrium occurs approximately on day 15 of pregnancy (Figure 4-2), and measurement of the uterine cavity is possible from day 20. Confirmation of the embryo (fetal sac) can occur on approximately day 33 (Figure 4-3), the fetal heartbeat can be confirmed on approximately day 54, and fetal biparietal diameter can be measured on approximately day 80 of pregnancy (Figure 4-4).

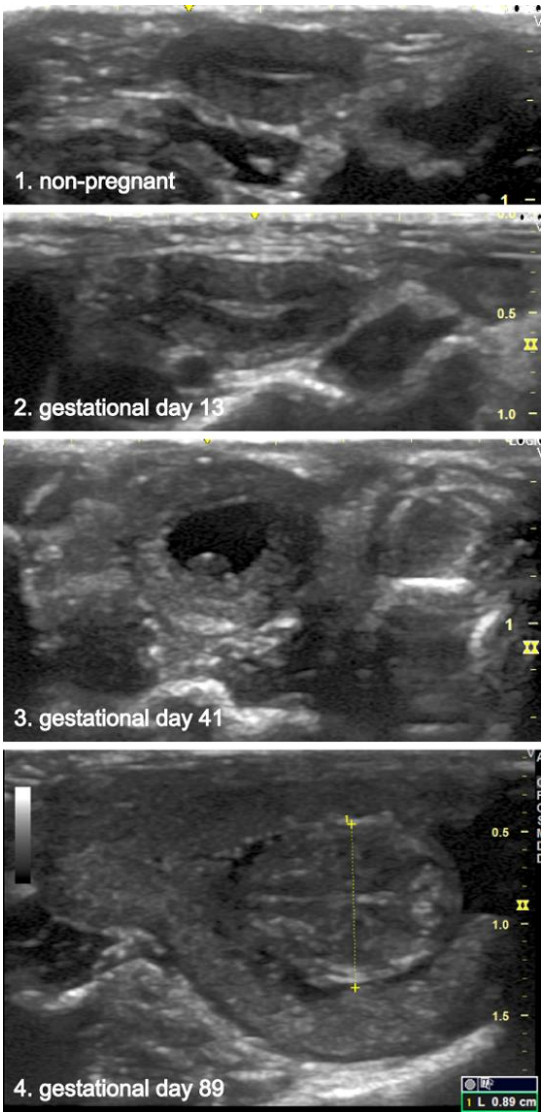

Figure 4 Ultrasound images of the common marmoset uterus; 1: non-pregnant, 2: gestational day 13, 3: gestational day 41, 4: gestational day 89 (dashed lines indicate fetal biparietal diameter: BPD). Estimated pregnancy days are calculated assuming ovulation at 10 days after luteal regression (delivery, abortion).

(2) Nutritional support for mothers

Common marmoset embryos develop slowly until approximately day 60 of pregnancy and rapidly grow after day 90 with the completion of placentation (Riesche et al., 2018). The energy intake of the common marmoset does not increase markedly during pregnancy (Nievergelt & Martin, 1999). However, nutritional deficiencies in the mid-pregnancy period (days 60–100) can lead to abortion when the fetus grows actively and the placenta is completely developed (Tardif & Bales, 2004). Abortions in our colony also occurred 80–90 days before and after placental completion. For pregnancy continuation and healthy

fetal development, nutritional or well-balanced food, such as enteral nutrition and supplemental foods arranged with solid feeds, must be provided to mothers after the period of mid-pregnancy. The nutritional supplements used in this study are listed in Table 2. Mothers should be weighed at least once a month during pregnancy to check for abnormalities.

(3) Delivery

After delivery, newborns are captured to determine their sex and weight. Whether they are properly clinging to the parent is confirmed, and any issues with movements, faces, and calls are checked. The mother's weight is measured, and if possible, subcutaneous fluid is used to rehydrate (4 mL acetic acid Ringer's solution + 0.1 mL vitamin B12 + 0.05 mL cyanocobalamin/head). Oral administration of fluid (Sorita T3 granules 0.4 g/10 mL water, 3–4 mL/dose) is also effective. If bleeding is excessive, vitamin supplementation may be beneficial.

(4) Postpartum

Postpartum monitoring: During the approximately 3-month lactation period, particularly in its latter half, the mother's energy intake increases 1.5–2 times (Nievergelt & Martin, 1999). Pre-weaned animals, dependent on maternal milk, are more susceptible to the mother's physical condition. Therefore, mothers' nutrition should be supported more carefully during lactation. Newborns' weight was measured regularly to confirm their daily weight gain. According to the reports from Europe and the United States, where the average weight of adults is 400 g, the early and later growth rates from birth to 5 months and 5 to 12 months of age are 1.15 g/day and 0.83 g/day, respectively (Schultz-Darken et al., 2016). How large they grow within 12 months is crucial for raising well-built marmosets. The target increase in weight is 1

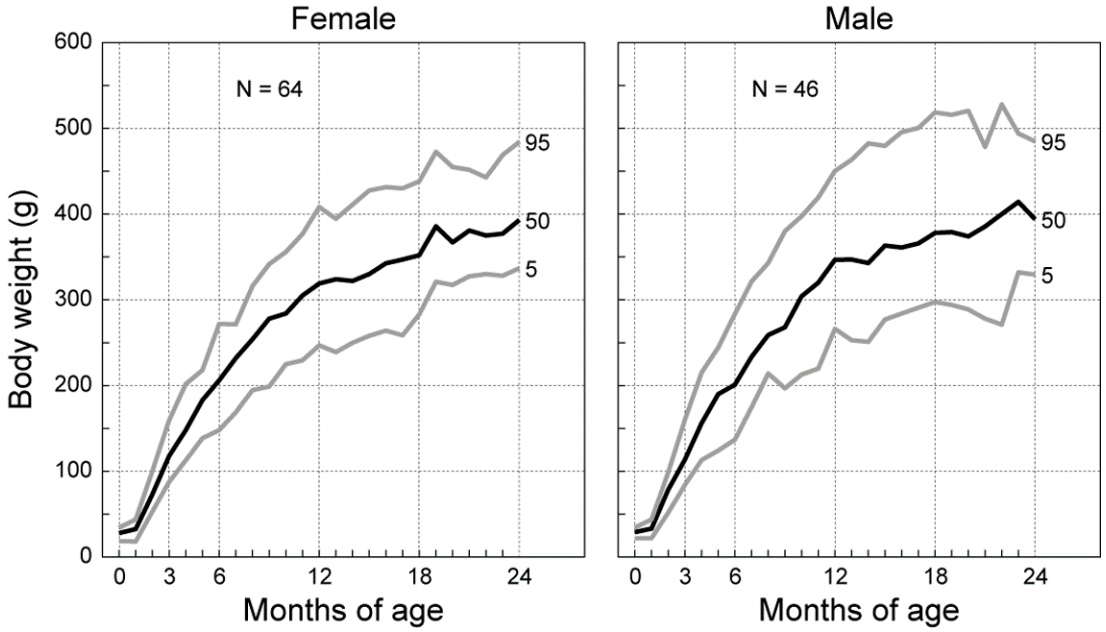

Figure 5 Body weight growth curves of common marmosets (5, 50, and 95th percentile curves). Data from 110 individuals (46 males and 64 females) born since January 2017. Data from the following individuals are excluded: Those who underwent artificial rearing, those who were experimentally used, those with spinal cord injury or leg transection by trauma, and breeding females used from the day of ovulation to the day of delivery.

g/day, with supplementary milk and food less than that (Table 2). Figure 5 shows colony growth curves.

Birth control: Established pairs are maintained in cohabitation for as long as possible (Poole et al., 1999). In captivity, nutritional well-being increases the number of offspring, placing a substantial burden on breeding females (Tardif et al., 2008). Birth control measures should be implemented to prevent maternal exhaustion. Birth control used in common marmosets includes "contraception," to avoid ovulation by administration of sex hormones, and "early abortion," to regress the corpus luteum by administering prostaglandin F2α (PGF2α) analogs (EAZA, 2017). In our colony, we implement "early abortion" to avoid disrupting the endocrine system. Common marmosets are highly sensitive to PGF2α, and the corpus luteum reliably regresses the day after administration; however, their normal reproductive cycle is maintained (Nievergelt & Pryce, 1996). Thus, births should be adjusted according to a female's health status.

[2] Feeding

1. Feeding method

In the wild, common marmosets exhibit activity peaks in the morning and afternoon, aligning with their foraging behavior (Castro et al., 2003). Even under captive conditions, where a diet is provided without the need to forage, small frequent feedings are desirable, given their activity patterns in the wild and the burden on their digestive systems. Feeding should be divided into morning and afternoon. Herein, we briefly describe the feeding process. For more details, see other studies (EAZA, 2017; Power & Koutsos, 2019; Goodroe et al., 2021).

The daily caloric requirement of common marmosets is approximately 50 kcal for adults (Power et al., 2019). Considering losses during feeding, approximately 1.5 times this amount should be provided. Animals with higher energy demands, such as juveniles and breeders, require special attention. Feeding boxes should be placed judiciously at the top of the cage, with perches or steps at the installation site. In cases of housing multiple animals together, measures such as adding more feeding boxes, placing them in multiple locations, providing slightly more to account for increased losses, and installing shallow boxes should be carried out to be accessed by young animals.

2. Diet

Wild common marmosets primarily consume exudates oozing from damaged trees, with a reported food composition of 68.5% exudates, 24.3% fruits, and 6.9% animal-based feed such as insects (Amora et al., 2013). According to the EAZA Best Practice Guidelines for Callitrichdae (EAZA, 2017), the recommended diet composition for marmosets that consume exudates is complete feed (solid feed), 15%; vegetables, 45%; insects, 15%; gum, 20%; and fruit, 5% (as a reward). They also suggested providing multiple types of vegetables and fruits. However, when kept as experimental animals, it is best to provide them with a diet primarily consisting of solid feed that conforms to the nutritional recommendations of the National Research Council in the United States (National Research Council, 2003) (Goodroe et al., 2021). Focusing on solid feed in most diets ensures a sufficient supply of macronutrients (carbohydrates, proteins, and fats) and micronutrients (vitamins and minerals). In cafeteria-style feeding (a feeding method that provides various supplements

simultaneously with the main diet), caution is needed as supplements might be preferred over the main diet, leading to inadequate nutritional intake (Power & Koutsos, 2019). Additionally, as common marmosets are an exudate-feeding species, gum feeding is strongly recommended. Commercially available sweet fruits, marshmallows, castella, and other confectionery items should not be used as feed.

Solid feed: The solid feed is dry. Juveniles, older animals, or those with difficulty chewing because of oral conditions may need moistened and softened feed. It is advisable not to soften it excessively while preserving the shape of the solid feed to maintain the oral health of animals and microbial hygiene in feed.

Gum: Gum arabic is commonly used in captivity. Gum arabic is an exudate collected from *Acacia Senegal* (Senegal species) and *Acacia Seyal* (Seyal species), belonging to *Acacia*, Fabaceae. Common marmosets showed a high preference for gum from the Senegal species. This preference is attributed to the similarity of the gum from *Parkia* pods (gum from *Parkia*, Fabaceae) consumed by common marmosets in their natural habitat, which has similar polysaccharide components (Peres, 2000). Gum arabic is available as a food additive and feed ingredient in various forms, such as chunks, spray-dried powder, and granules. Chunks of gum can be prepared as a whole, whereas powders or granules can be dissolved in water. Mixing them with powdered solid feed, nutritional supplements, and probiotics results in good supplementation (Table 2).

Supplements: Insects and vegetables are recommended (EAZA 2017). Providing mealworms is also

Table 2 Supplementary feedings and enteral nutrition

Supplementary feedings	Ingredients/Components		
Mealworms	*Larva, pupa, adult*		
Mashed feed	Solid feed powder + gum arabic + water		
Solid feed gum	Gum arabic + solid feed powder + probiotics preparation + infant formula + protein + water		
Solid gum	Gum arabic + water (small amount)		
Liquid gum	Gum arabic + water		
Arabic gum crystals	Gum arabic crystals		
CMS Jelly	CMS jelly powder + water		
Scrambled Eggs	Heat eggs in a microwave		
Sweet Potato	Raw, boiled, dried		
Steamed rice	Rice + probiotics preparations + gum arabic		
Nutrition	**Type**	**Manufacture**	**Compounding**
ELENTAL®P combination powder	Elemental diet	EA Pharma	1.5 g + 5 mL water
Tube Diet® <High- Calorie/High Protein>	Oligomeric formula	Morinaga Sunworld	1.6 g + 5 mL water
Isocal® 1.0 Junior	Polymeric formula	Nestlé Health Science Company	
Morinaga E-Akachan	Infant formula	Morinaga Milk Industry	1.3 g + 10 mL hot water
GFO®	intestinal supplement	Otsuka Pharmaceutical	0.5 g + 5 mL water

beneficial since they considerably prefer the adult stage, larvae, and pupae. “Mashed feed,” the solid feed powder solidified with gum arabic, can also be given depending on the individual’s condition. The feeding amount of supplements not containing solid feed should not exceed 10% of the caloric intake (4–5 kcal/day) (Power et al., 2019).

[3] Housing environment

1. Cage

For detailed information on the housing environment, please refer to other studies (Lazaro-Perea et al., 2000; Japan Neuroscience Society, 2021). Only the key points are mentioned here. Because common marmosets are arboreal animals, cages with sufficient height are preferred. Cage accessories should be appropriately arranged to induce natural behaviors, such as jumping between tree branches, running, hanging, gnawing on trees, resting on branches, and sleeping in enclosed spaces (tree hollows).

Perches: Perches are necessary for moving, resting, marking, and gnawing during movement. Common materials include fruit trees (pears and apples), cedars, spruce-fir-pine lumber, and whitewood.

Nesting box: It is necessary for sleeping and hiding. Preferably placed at a high position, the entrance should be of a size (diameter, 8–10 cm) that allows an adult carrying a newborn to pass comfortably. Wooden nesting boxes can also serve as gnawing material.

Gnawing wood: In addition to perches, it is also beneficial to have wood specifically for gnawing. They prefer softer wood to harder wood (Thompson et al., 2014), with balsa wood being particularly preferred. It helps prevent dental plaque and tartar build-up, aids in stress relief, prevents self-injuring behaviors, and minimizes tampering with injured areas.

Other: Swings, hammocks, and steps are also beneficial. Paper products such as cardboard boxes, cardboard tubes, and bags of solid feed are excellent tools for chewing (Jennings et al., 2009). However, care must be taken with extremely thin ropes or fabric products, as there is a risk of accidents in which the rope or sewing thread may entangle the body or neck. Additionally, for young or unhealthy animals, placing warming pads above the cage or using resin nets on metal floors may be helpful.

2. Number of animals

In the wild, common marmosets live in family groups consisting of parents and offspring; desirably, this family should be retained with parents and 2–3 pairs of twins of different ages.

Younger animals need to care for their siblings during their developmental period. A lack of such experience may lead to difficulties raising offspring when they become parents themselves (Tardif et al., 1984; Lazaro-Perea et al., 2000). European standards recommend keeping animals intended for future breeding within their natal family units until at least 13 months of age; it is desirable to keep them within their families as much as possible (EU Commission recommendations, 2007; EAZA, 2017; NC3Rs, 2017). They should not be separated from their families until at least one year of age if they are not offered for breeding.

[4] Daily observation and health management

Daily observations are crucial for establishing well-built common marmosets. Owing to their small size, once their health deteriorates, it can quickly lead to life-threatening conditions. Rapidly recognizing subtle changes in their condition and appropriately responding is imperative for successfully breeding well-built marmosets. The following points discuss the items to be observed daily and important considerations.

1. Environmental observation

For temperature, humidity, and other factors that can be quantified, the daily maximum and minimum values must be recorded in addition to the current values. Special attention should be paid to temperature as it significantly affects animals. Marmosets require energy to maintain a constant temperature if the room temperature is below 27°C (Power, 1991). The temperature in each cage may have differed from that of the thermometer. The temperature gradients at various locations must be determined, and the temperature where the animals are located must be estimated based on thermometer readings. The changes in ventilation frequency, brightness, noise, and vibrations must be noted, and proper water and feed supply must be ensured.

2. Individual observation

The Common Marmoset Care website at the University of Stirling presents important points regarding behavioral patterns (ethogram), facial expressions, postures, and call types. The following points should be learned for basic knowledge: individual factors, such as age, sex, constitution, personality, and medical history, should be considered, and the normal state of each individual while being vigilant for abnormalities should be understood. During the observations, they stood in front of the cage to observe the animals' free behavior and noted their reactions when approaching and moving away from the cage; responses to interactions, such as when humans greet and present food, are observed.

(1) Observation frequency

Observations should be made twice a day, in the morning and afternoon. Morning observations should be performed as early as possible, and afternoon observations should be conducted after afternoon feeding. Even if animals appear active during the day, the following behaviors indicate early signs of health issues: signs such as inactivity in the morning; initiation of movement only when humans enter the room; initiating movement only when approaching in front of the cage, and promptly retreating from the nesting box after routine events for the day.

(2) Observation points

Individual states, such as appearance, activity level, or falling objects originating from the individual, such as vomiting, should be observed. In group housing, we confirmed the relationship between group members.

Physical conditions

Posture: As animals become unwell, they tend to seek warmer places, contract their bodies, attempt to hide, lower their heads, lie face down, and eventually lose their ability to maintain their posture and lie down.

Table 3 Checklist of observation points for individual condition

Section	Checklist
Posture	Are they in a warm place, such as the top of the cage or on a warm pad, or in a place where they can hide, such as a nesting box? Are they stretching? Is their tail weak? Is the tail curled up, and if so, is it curled up only at the tip of the tail, or as a whole and tucked between the hind legs and abdomen? Are they sitting or lying down? Is the head down?
Activity	Where do they stay in the early morning, and do they hide in the back of the cage? Whether they respond to human entry or proximity to the cage? Whether they respond to the presentation of food? Whether they move as usual or whether the amount, frequency, and speed of their activity decrease. Whether there any laterality in their movements? Are there any diurnal variations or patterns in their movements?
Face	Can you make eye contact? Is there power in their eyes? Are the eyelids drooping? Are the eyelids completely closed and are there any laterality? Is there any abnormality in the position of the pupil? Is the pupil size normal and no laterality?
Coat	How about the condition of the tailcoat, especially at the root or ventral part of the tail? Is the stripe pattern on the tail preserved? Is the ear hair coarse, parted, short, or are the dark lines visible? Is the coat in the groin and lower abdomen? Is the coat sticky? Is the coat color of the head (cape area)? Is there any hair loss? How about the condition and quantity of shedding hair in the cage and floor?

Additionally, the “stretch” behavior, where animals grasp the cage with both hands or feet, elongating their trunk, has been reported as an indicator of a problem with the gastrointestinal system (Ross et al., 2012). The observation points are listed in Table 3.

Activity level: Animals tend to be less active when they are unwell. Signs included: 1) Not coming to the front of the cage; 2) not grasping vertical surfaces such as cage bars or perches; 3) staying on the cage floor or step where they could sit; 4) retreating to the nesting box; 5) reduced movement quantity, frequency, and speed; and 6) not responding to humans or food presentations. Overall activity decreases in cases of internal medical issues such as diarrhea. In cases of traumatic injury, only the affected part exhibits reduced movement and unbalanced behavior. Morning activity decreases in conditions such as anemia, hypoglycemia, or malnutrition. In the early stages of illness, activity may only decrease when humans are absent or during events such as feeding, and the individual may retreat to the nesting box early in the evening. The observation points are listed in Table 3.

Facial expression: As animals become unwell, their eyes lose power, eyelids droop and become hollow, and eye contact becomes difficult. Abnormalities in the nervous system include abnormal eye movements, pupil size or position, and eyelid closure. The observation points are listed in Table 3.

Calls: When chatter, Ek, Tsik, or submissive (distress) are heard, the situation should be checked and the animal should be taken care of, or the environment should be adjusted as needed. Submissive calls

are similar to infant cries (babbling or ngã) emitted by infants or juveniles and are produced when subordinate animals face dominant animals or experience aggression or threat (Snowdon, 2017). In addition, exercise caution if Trill, Chirp, Twitter, or Phee are completely inaudible as they may indicate an abnormality.

Coat: As animals become unwell, the coat, particularly around the bottom of the tail and ventral part below the anus, often becomes coarse. Coat loss is also common in these areas. The thickness of the tail and the presence of stickiness reflect the well-being of the animals. If the coat of the tail is thin and sticky, it indicates illness. In younger animals, the tail condition is an indicator of growth. Ear hair becomes coarse and short or appears as a black line in the ear tufts. For lactating females, after approximately 2–3 months postpartum, changes in ear hair are often observed. Whether the auricles (particularly the upper part of the ear tuft) are visible or transparent should be checked. In addition, the coat is observed in areas such as the inguinal region, lower abdomen, and around the clavicle, and unhealthy animals may have a thin coat in these areas. The appearance of the marmoset coat varies by body part, with the head being uniformly black, the trunk exhibiting a ring-shaped gradient owing to the coat being in three colors (black, orange, and grey) from the root, and the tail showing black and grey stripes (Hershkovitz, 1977; Stevenson & Rylands, 1988). When unwell, coat shedding disrupts these patterns, making the colors pale. The head (cape area) is particularly useful for determining the extent of fading because it is uniformly black. In lactating females, at approximately 2–3 months postpartum, line-shaped faded areas may appear around the face (top, bottom, left, and right). The observation points are listed in Table 3.

Skin: Commonly observed abnormalities include facial redness, swelling, and fistula (external dental fistula) owing to trauma or periapical abscess, as well as dehydration (decreased turgor) and sudamina (heat rash) in neonates during the rainy season.

Body condition (fleshiness): If unwellness or loss of appetite persists, fat and muscle mass diminish, leading to emaciation. If one recognizes that an adult has lost weight, the individual often decreases their weight by 10–20 g or more. In animals with a good body condition and have fat deposits (fat pad) in the axilla, wrinkling of the axillary skin because of fat consumption may be an indicator of their weight loss. As emaciation progresses, the inguinal region of the inner thigh may become concave, allowing visualization of the femoral vein.

Natural orifices: The state of the natural orifices (eyes, nostrils, external acoustic foramen, mouth, anus, urethral orifice, and vagina) should be assessed; exudates or bleeding should be checked. If there is an exudate, its nature (serous, purulent, mucous), quantity, and timing (only in the morning) should be determined.

Abdominal distension: The abdomen is distended owing to gastrointestinal obstruction, duodenal dilation, or abnormal fermentation in the gastrointestinal tract. Common marmosets often experience gastrointestinal system abnormalities and are attentive to the presence of abdominal distension after understanding their normal states.

Falling objects

Feces: During the morning and afternoon observations, the shape, color, odor, quantity, size, and defecation patterns are recorded. Multiple animals from each cage are evaluated in a single group. However, if severe or persistent diarrhea, constipation, or decreased food intake is observed, the affected individual can be identified and temporarily isolated.

(Shape) It is evaluated based on water content, ranging from hard, normal, soft, muddy to watery feces. Hard or watery feces cases requiring prompt intervention need attention.

(Color) Normal feces are brown; however, color changes can occur owing to indigestion, enteritis, diarrhea, or disruption of the intestinal flora. If digestion and absorption are insufficient, feces may become yellowish-white, increasing fat excretion. With an increased starch content, they may take on an orange tint. Green feces may also be observed in animals with advanced gastrointestinal symptoms because of the increased or inhibited reabsorption of bilirubin and urobilinogen. Green feces can also occur in cases of malnutrition. If the moisture content of the green feces is low or dry after defecation, it may turn dark green or black. If the blood is red, it determines whether it is only adhering to the surface of the feces, mixed throughout, or mixed with mucus. In cases of upper gastrointestinal tract bleeding, the feces may appear black.

(Odor) Generally, bacterial diarrhea and fatty feces during indigestion produce a strong, acidic smell.

(Quantity) The normal amount of feces for each individual and whether it is more or less must be determined. Immediate intervention is required in cases of no feces or a large amount of diarrhea (muddy to watery). In the absence of feces, there is a risk of bowel obstruction.

(Size) Extreme sizes, either too large or too small, require attention. If the feces are thicker and larger than usual, this may suggest chronic lymphocytic colitis, common in marmosets. If small, factors such as increased reabsorption of fecal moisture due to reduced peristalsis, decreased water intake, reduced food intake, reduced activity, decreased abdominal muscle strength or masses owing to tumors should be considered.

(Defecation pattern) The timing and properties of defecation should be determined. For instance, if loose feces and diarrhea occur during the day and normal feces occur from the evening to the next morning, it suggests an imbalance between the feeding amount and content as a digestive burden for the gastrointestinal tract.

Vomiting: Property, quantity, and timing should be confirmed. Vomiting should be checked under or inside the cage and around the mouth and throat.

(Property) Vomiting may consist of crushed food, saliva, gastric fluid, blood, and bile. If it is difficult to distinguish whether it is expelled from the oral cavity, ejected from the esophagus, or vomited with stomach contents, the smell of the vomiting is checked. If there is an acid smell, it might be “vomit.” Abnormalities in the teeth or tooth ridges can lead to saliva adhering from the mouth to the throat. If vomiting occurs from the gastrointestinal tract below the stomach,

gastrointestinal tract passage disorders, such as intestinal obstruction or duodenal dilation, are suspected.

(Quantity) Often, small doses cause vomiting, and it is challenging to distinguish properties over time. In particular, only gastric fluid must be directly observed for diagnosis. Continuous vomiting, even with small doses or with abundant vomiting, requires immediate attention.

(Timing) Observation over time may be sufficient when vomiting is a single episode without any activity or appetite abnormalities. However, prompt intervention is necessary for other symptoms such as lethargy, sudden loss of appetite, or constipation because of the possibility of intestinal obstruction.

Feed: The amount of food remaining in the feeding box (remaining food) and the amount that has fallen under the cage (spillage) are checked during daily observation. The amount consumed (intake) is determined by subtracting the remaining spillage from the feed amount. Spillage decreases as individual activity decreases to explore or touch the food when appetite is lost.

Blood stains: The quantity, properties, and location of bloodstains are observed. Rectal bleeding, blood vomiting, nosebleeds, bleeding from gums, or injuries are distinguished, and responses are given accordingly. There is a possibility of genital bleeding or postpartum discharge in breeding females.

Coat: Malnutrition increases shedding, and fur may adhere to the cage pitch or bottom of the cage.

Relationship with family members

When housing multiple animals together, changes in their relationship with the family members indicate unwellness. Malabsorption or low nutrition leads to increased greed for food, intimidation, or aggression towards family members or caretakers during feeding. Family members may take shelter with unhealthy animals. Immediate intervention is required in such situations. Stress owing to the deterioration of relationships with family members can also lead to health issues. Behaviors indicative of stress, such as avoiding contact with specific animals, avoiding proximity, being chased or attacked, emitting submissive calls to specific animals, self-biting or coat pulling, increased marking, and scratching with the forelimbs should be paid attention. On observation of suppressed stress, separation is considered.

3. Response to abnormal detection

On observing abnormalities in an individual, whether immediate veterinary treatment is necessary or whether follow-up observation with nutritional supplementation or environmental improvement is sufficient should be determined first.

(1) Urgent venous treatment

When the following symptoms are observed, promptly contact a veterinarian: lying down; prone; sitting with the head lowered; depression; closed eyes; slow movements; lameness (inability to walk normally); visible injuries; bone fractures; severe diarrhea; mucus bloody feces; rectal bleeding; vomiting blood; hemorrhage; severe constipation; decreased urine volume; eating deprivation; complete loss of appetite; labored breathing (raising shoulders and appearing difficulty with each breath); and coat

strangulation of the penis. Simultaneously, the following is performed: the animal's condition and situation with photographs or videos are documented; diarrhea or vomiting samples are collected; the animal's weight and body temperature are measured; the animal is kept warm; food is removed during preparation for anesthesia; and dehydration and rehydration are checked if instructed.

(2) Cases not urgent; however, requiring careful observation

Mild activity reduction, decreased food intake, loss of appetite, mild vomiting, mild diarrhea (muddy feces), coat shedding on the tail or ears, weight loss, mild injuries, sneezing, nasal discharge, and other mild respiratory symptoms are observed cautiously. Simultaneously, the following are performed: weight is measured; a warm place with options such as a heating pad or lamp is provided; nutritional supplementation and rehydration are provided; changes in feeding content and methods are noted; and anticipated stress is eliminated with adjustment to the environment.

Conclusion

Many animals in the colony have experienced a decline in their health. The manual was compiled based on more than 15 years of experience. We anticipate that individuals involved in marmoset care will implement this manual into their care environment and find it advantageous as a quick reference when needed. We hope that a healthy and well-built marmoset population will continue to grow in Japan.

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