NOTE *Laboratory Animal Science*

Congenital malformation of the vaginal orifice, imperforate vagina, in the common marmoset (*Callithrix jacchus*)

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ABSTRACT. The following is a report on a congenital vaginal malformation, imperforate vagina, in the common marmoset (*Callithrix jacchus*). This anomaly was observed for the first time in an adult female in our research colony. There was no uterine and vaginal aplasia or atresia in her grossly normal genital tract. The plasma progesterone concentration suggested that the ovarian cycle had ceased. However, this may not be related to a functional anomaly, but rather to suppressed ovulation resulting from subordination to cagemates considering the various stages of follicular development observed.

KEY WORDS: common marmoset, congenital malformation, imperforate vagina, ovarian cycle

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The common marmoset (*Callithrix jacchus*) is a small New World primate native to Brazil, increasingly used as an alternative primate model in biomedical research areas including reproduction, neurobiology, immunology, endocrine signaling, obesity, aging and fetal or postnatal development [4, 13]. Recently, a transgenic marmoset line was established with germline transmission [19], attracting attention to the potential for this animal model.

There are approximately 200 marmosets, including 20 breeding pairs, in our research colony. Uterine size, as determined by palpation, has been used to estimate the gestational age. However, this method is affected by factors, such as litter size, individual differences in uterine size (parous vs. non-parous) and operator skill. Thus, in our facility, the plasma progesterone concentrations in females paired with males are monitored weekly to estimate the gestational stage and delivery date. Additionally, unmated adult females have plasma progesterone levels monitored at 2–3 day intervals in preparation for pairing. An imperforate vagina was observed among one of these unmated females. Congenital malformations of the external genitalia in the common marmoset have rarely been reported, with the exceptions of fused labia [11] and imperforate vagina [17]. Isachenko *et al.* reported fused labia found in the offspring from 10 marmoset pairs, one or both of which were introduced to their facility, the German Primate Center, from 2 genetically related colonies in Munich, Germany and from one colony in Basel, Switzerland. This abnormality appeared to be recessive and heritable [11]. In Japan, common marmosets are obtained from breeders, interfacility transfers or through in-house breeding programs. Japan does not allow the importation of common marmosets from European countries, including Germany and Switzerland. Therefore, our colony does not include any individuals related to the colonies from which Isacheko *et al.* purchased the fused labia carriers. Here, we report a case of imperforate vagina detected in our colony that occurred despite the avoidance of inbreeding. The research was conducted in accordance with the Declaration of the Helsinki and was approved by the Animal Experiments Committee of RIKEN (Wako, Japan). All animals were cared for and treated humanely in accordance with the Institutional Guideline for Experiments using Animals (Approved ID: No. H25-2-212 and H25-2-219).

The external genitalia of the adult females are shown in Fig. 1. The individual with the imperforate vagina (254F) was born in our facility and was 24 months of age at the time of dissection. There was a sulcus in the center of the vulva (Fig. 1a), grossly similar to the morphology of normal females (275F and 318F, Fig. 1b and 1c, respectively). Upon spreading, the labia were found to be fused (Fig. 1d), unlike those of the normal females (Fig. 1e and 1f). The morphologically normal females, also born in our colony, were 23 and 20 months of age at the time of dissection. In addition, 318F was a sibling of 254F.

The female genital tracts, including the external vulvar skin (Fig. 2a), were dissected after perfusion with saline followed by 4% paraformaldehyde under deep anesthesia. They were fixed using tissue fixative (Genostaff, Co., Ltd., Tokyo, Japan), embedded in paraffin and cut into 6-μm sections for hematoxylin and eosin (HE) staining. Aplasia or atresia of the uterus and vagina was not observed in the genital tract of 254F (Fig. 2b), which was grossly similar to those of 275F and 318F (Fig. 2c and 2d, respectively), except for the imperforate vagina. The vaginal orifice was closed in 254F (Fig. 2e), whereas those of 275F and 318F

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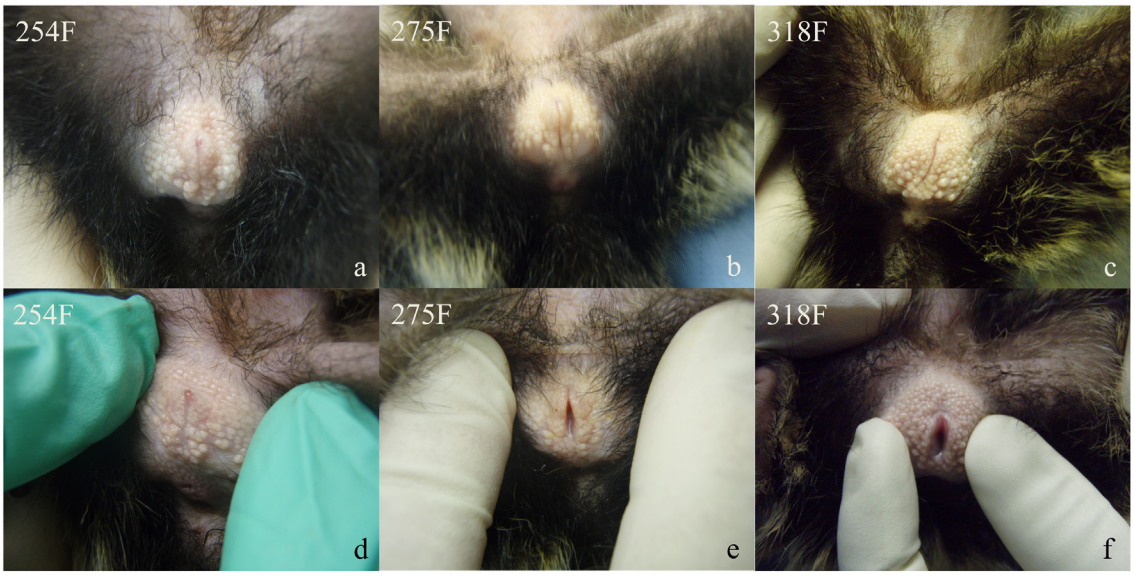

Fig. 1. External female genital morphology. There was a sulcus in the center of the vulva in 254F (a) similar to the normal females, 275F (b) and 318F (c). Spreading showed that the labia of 254F were attached (d), unlike those of 275F (e) and 318F (f).

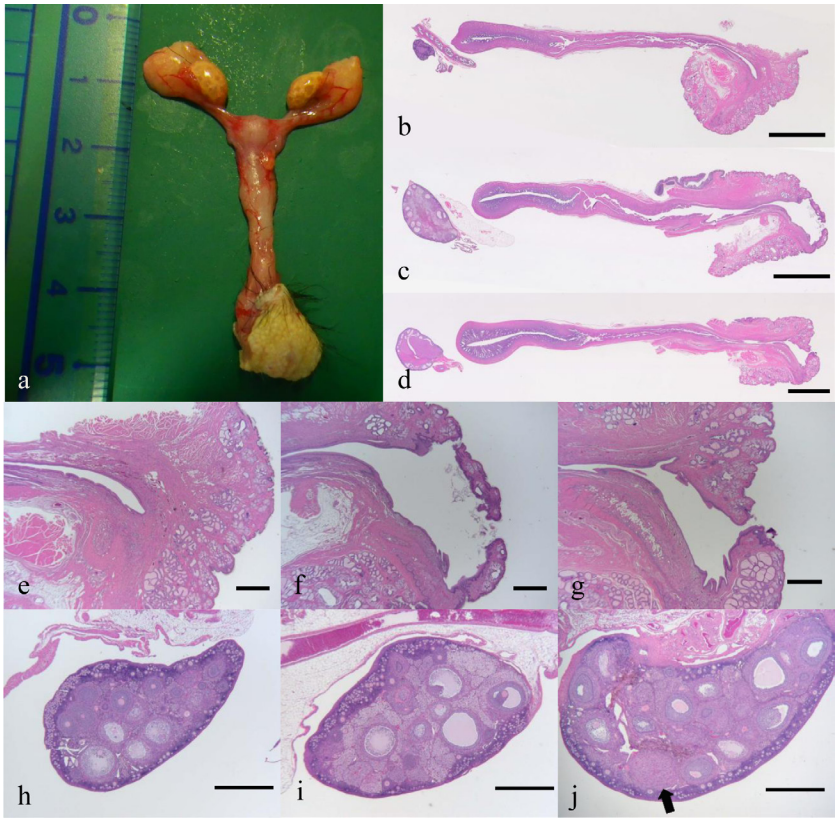

Fig. 2. Female genital tract morphology. The genital tract with the vulvar surface skin (a) was dissected after perfusion with saline followed by 4% paraformaldehyde under deep anesthesia. Aplasia or atresia of the uterus and vagina was not observed in 254F (b) and appeared grossly similar to those of 275F (c) and 318F (d), with the exception of the imperforate vagina. The vaginal orifice in 254F was closed (e), whereas those of 275F (f) and 318F (g) were open. Various follicular stages, including mature follicles, were observed in 254F (h), similar to 275F (i) and 318F (j). This suggests that 254F had the capacity for normal follicular development. Serial sections of the entire ovary did not show a CL in 254F or 275F, whereas a CL was observed in 318F (j). The CL was indicated by an arrow. Bar: 5 mm (b–d), 1 mm (e–j).

Figure 3a

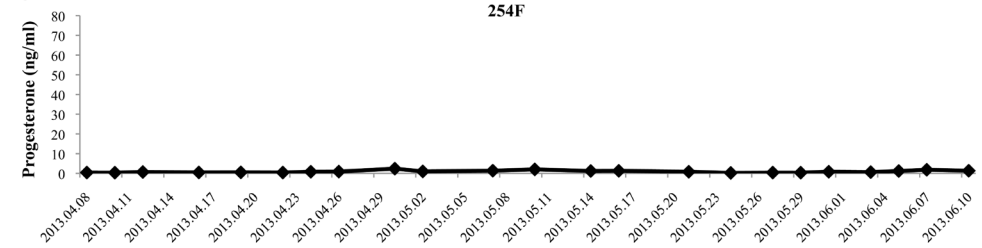

Figure 3b

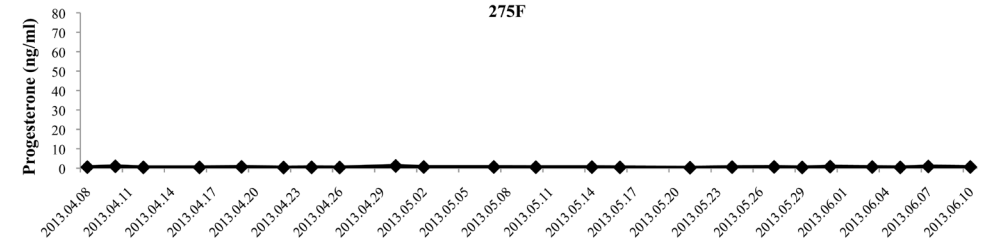

Figure 3c

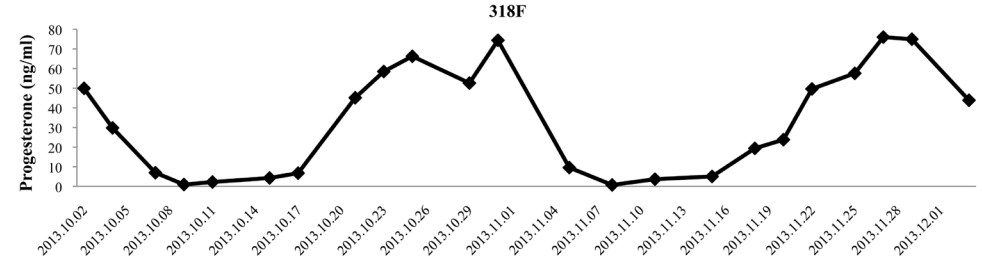

Fig. 3. Plasma progesterone concentrations in 254F (a), 275F (b) and 318F (c). Ovarian cycling was absent in 254F and 275F, whereas 318F displayed a normal cycle.

were open (Fig. 2f and 2g, respectively). Both of the vulvar skin and subcutaneous glandular tissue (glandulae vestibulares minores) are seamless in 254F (Fig. 2e). In addition, the dead end of the vaginal squamous epithelium layer is also observed (Fig. 2e). These observations suggested that the closed vaginal orifice of 254F was not a case of synechia, but a congenital malformation. In contrast, the labia were joined by a thin band of fibrous tissue in the case reported by Isachenko *et al.*, suggesting a different mechanism. Mayer-Rokitansky-Kuster-Hauser syndrome (MRKH), a human congenital disease causing aplasia or hypoplasia of the uterus and the upper part of the vagina [15], has an estimated incidence of 1 in 4,000–5,000 female births [9, 10]. However, the lower third of the vagina is always present in MRKH patients [16, 20]. Aplasia or hypoplasia of the uterus and upper vagina was not observed in 254F, indicating the pathogenesis may differ from that of human MRKH. In humans, the differentiation of the female external genitalia is a default process that occurs as a result of the absence of dihydrotestosterone (DHT) during the ninth and twelfth weeks of intrauterine life [14]. In the absence of DHT, the urogenital and labioscrotal folds become the labia minora and majora, respectively [14]. As congenital external anomalies of the female reproductive tract in common marmosets have been described rarely, the mechanisms involved are unclear. An error may have occurred during external genitalia differentiation in 254F. The ovaries of 254F were small in size (Fig. 2h) compared with the mean size (5.3 × 4.3 × 3.8 mm) in normal adult common marmosets [7]. However, various follicular stages, including mature follicles (Graafian follicle), were observed in 254F (Fig. 2h). The ovaries were similar to those of 275F and 318F (Fig. 2i and 2j, respectively), suggesting that 254F had normal follicular development. Serial sections of the entire ovary did not reveal any corpus lutea (CL) in 254F or 275F, while a CL was found in 318F (Fig. 2j). These findings suggest that ovulation was suppressed, despite normal follicular development in 254 and 275F, which corresponds to the plasma progesterone levels (Fig. 3a and 3b, respectively).

The circulating plasma concentrations of progesterone seen in 318F were characteristic of the follicular and luteal phases of a normal ovarian cycle (Fig. 3c); 254F and 275F showed only follicular-phase progesterone concentrations, indicating anovulation (Fig. 3a and 3b, respectively). Social status (dominant or subordinate) among adult females may suppress ovulation. In captive and free-living common

marmosets, usually only a single dominant male and female breed [4]. All other group members (offspring of the dominant pair or unrelated animals) aid in rearing infants born to the dominant pair [4]. This cooperative breeding system occurs through inhibition of sexual behaviors and neuroendocrine inhibition of ovulation in subordinate daughters and females unrelated to the dominant breeding female [4]. Suppressed ovulation in subordinate females has been reported in captive colonies of common marmosets [1–3, 5]. Both 254F and 275F were housed with other females, and each cagemate showed a normal ovarian cycle (data not shown). Therefore, ovulation in 254F and 275F may have been suppressed as a result of subordination to their cagemates.

Isachenko *et al.* suggested that fused labia are recessive and inheritable [11]. In addition, in pairs in which both animals were from suspicious colony, 45% of the female offspring had vulvar abnormality [11]. In contrast, 2 female siblings of 254F, including 318F, had normal vulvar morphology. In addition, except for the case of 254F, imperforate vagina has not been observed in our colony or other related individuals. Since 254F had few female siblings, we were unable to determine whether this is an inheritable phenomenon or a sporadic anomaly.

Since they live in family groups and all members aid in infant rearing [4], common marmosets have been used in familial sociality research [8, 18]. As common marmosets have similar sex hormone profiles to those of the human ovarian cycle [21], they are often used in reproductive research and developmental engineering [6, 12, 19, 22]. Infertile females, including those with imperforate vagina, cannot be used for experiments in these fields. This has a negative impact on the use of this species as a laboratory animal model. We hope that the present report familiarizes researchers with this congenital malformation in the common marmoset. Imperforate vagina can occur, despite the avoidance of inbreeding. Further research is required to clarify the incidence and mechanism (s) of this anomaly.

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