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Abstract

[Research Title]

Ex situ conservation of endangered wildlife using germ cells

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[Abstract]

In this study, we developed basic breeding information for endangered wildlife species by integrating genomic and endocrinological information. Based on this information, breeding individuals were evaluated, and using the stored germ cells, assisted reproductive technologies were developed for the Tsushima leopard cat (*Prionailurus bengalensis euptilurus*) and the Okinawa rail (*Gallirallus okinawae*). We also contributed to the ex-situ conservation by providing these results to relevant organizations as reference information for other endangered wildlife species.

In sub-theme 1, we accumulated and analyzed information obtained from genetics, hormones, and behavior in order to develop basic information on breeding. The genetic diversity of captive and wild populations was compared, and a population viability analysis (PVA) in the wild was conducted to determine conditions for survival after 100 years. Genome sequence information was used to identify genes related to reproduction and found to be associated with reproductive performance. We detected the percentage of DNA methylation in the genome of captive individuals with known ages and created a highly accurate age estimation model. Based on fecal sex steroid hormone levels and behavioral observations, we evaluated the reproductive physiological status of all captive females in the breeding program. Activity changes and stress hormone concentrations were shown to be useful for predicting reproductive status and stress. This genomic and hormonal information was recorded in a database for each individual, which can be compared with the germplasm information in sub-theme 2.

In sub-theme 2, bio-banking of germ cells with information from sub-theme 1 was developed. A transportation system was established to ensure that testes and ovaries collected from dead individuals arrive at the laboratory within 48 hours for the Tsushima leopard cat and within 24 hours for the Okinawa rail. In the Tsushima leopard cat, testes were removed from 17 male animals, and sperm were collected from 5 adult animals and frozen or freeze-dried for preservation. The preserved sperm were shown to have sufficient fertilization ability. Some of the sperm were fertilized with in-vitro matured oocytes to produce fertilized eggs. Ovaries were cryopreserved for 10 individuals. In the Okinawa rail, sperm from

113 individuals were frozen and 7 individuals were freeze-dried. Some of the frozen sperm were used for artificial insemination, and it was confirmed that the eggs were fertilized. Ovaries from three wild-dead females were also removed and cryopreserved. The frozen sperm and ovaries were stably stored in the National Institute for Environmental Studies.

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