Approaches to Improve Artificial Breeding of *Eudyptes chrysocome chrysocome*

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Rockhopper Penguins, *Eudyptes chrysocome chrysocome*, are classified as vulnerable (VU) by the International Union for Conservation of Nature (IUCN) Red List 2017 of Threatened Species. The reproductivity of captive Rockhopper Penguins failed to rise in Japan, resulting in risk of decreasing captive population. Kaiyukan and Tokyo Sea Life Park carried out a study for the technical establishment of artificial captive breeding and the result is reported here.

In this study, fluctuation in calcium, triglyceride and inorganic phosphorus concentrations in the blood was monitored during breeding season from March to May. Mating behaviour was observed from nesting to incubation period through video recording. Using these two methods, the optimal timing for artificial insemination (AI) and day of egg laying were estimated.

AI was conducted using semen from male penguins from Tokyo Sea Life Park. Under physical restraint, samples were collected by exerting digital pressure at the genital papillae. The samples were then diluted with Beltsville (Sexton, 1977) and maintained at 4-10 ℃ or less for 5-6 hours while being transported to Kaiyukan where semen were injected into female penguins. DNA tests were conducted on blood samples to confirm the identity of the male penguin.

Calcium, triglyceride and inorganic phosphorus concentrations in the blood increased from six weeks before egg laying and peaked at four days prior to egg laying. The day of egg laying could be estimated by monitoring these changes. Based on the observation of mating behavior, the optimal timing for AI was estimated 5-10 days before egg laying. Upon conducting AI 7-10 days prior to egg laying, one chick hatched. AI was confirmed successful for this chick as the parent was the semen donor.

Following this attempt of AI using frozen semen, we will continue further studies with the aim to contribute to the conservation of this species.