THE EFFECTS OF TYPE I COLLAGENASE ON THE DEGELIFICATION OF CHIMPANZEE (*Pan troglodytes*) SEMEN PLUG AND SPERM QUALITY: IMPROVING GENETIC DIVERSITY OF ZOO CAPTIVE CHIMPANZEE

Jane-Fang Yu[1,#], Yu-Hua Lai[2,3,#], Tse-En Wang[2,3], Yu-Syuan Wei[2], Yu-Jia Chang[1], Sheng-Hsiang Li[6], Shih-Chien Chin[1], Radhika Joshi[2], Hui-Wen Chang[2,5], Pei-Shiue Tsai[2,3,4]

[1]Conservation and Research Center, Taipei Zoo, 30 Xinguang Road, Section 2, Wenshan, Taipei 11656, Taiwan.

[2]Department of Veterinary Medicine, [3]Graduate Institute of Veterinary Medicine, [4]Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd. 10617 Taipei, Taiwan

[5]Graduate Institute of Molecular and Comparative Pathobiology, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd. 10617 Taipei, Taiwan

[6]Department of Medical Research, Mackay Memorial Hospital, , No. 92, Section 2, Zhongshan N. Rd, 251 Tamshui, Taiwan

Introduction

Semen from the chimpanzee species becomes a colloidal solid after ejaculation. The formation of this copulatory plug is believed to prevent additional spermatozoa of subsequent mating events from accessing the ova. However, this naturally preserved strategy hampers the processes for sperm preparation.

Materials and Methods

Sixteen semen samples were collected from 4 individuals using artificial vagina. Type I collagenase was used for co-incubation with semen ejaculates. Sperm concentration, motility, morphology, viability were assessed manually or with computer assisted sperm analysis system (CASA). Immunofluorescent and immunoblotting against specific antibody were used to evaluation collagenase effects on sperm capacitation and acrosome integrity.

Results and Discussion

We showed that incubation of chimpanzee ejaculates with 0.1% type I collagenase efficiently and significantly ($p<0.05$) releases 2.7-fold more spermatozoa from the coagulated ejaculates, and this degelification process did not alter sperm morphology or viability; nor did it stimulate spontaneous capacitation or an acrosome reaction as assessed by tyrosine phosphorylation and peanut agglutinin stains; moreover, based on computer assisted sperm analysis assay, motility-related parameters remained similar to those of untreated spermatozoa. Moreover, we observed that post collagenase treatment, 2.5% glycerol as a cryoprotectant, resulted in better acrosome integrity and 7.8% glycerol maintained sperm motility better than that of 2.5% glycerol. Our results demonstrated for the first time that type I collagenase can be used to obtain a significantly higher number of spermatozoa from colloid chimpanzee semen ejaculate without affecting the physiological properties of spermatozoa, and these results are critical for the subsequent gamete development. Our results would benefit sperm preparation processes and cryopreservation efficiency per ejaculate. These results would also benefit the genetic diversity of the chimpanzee species, using sperm cells from less dominant individuals, and for achieving better pregnancy success in primates with significantly higher amounts of sperm for artificial insemination.