UPDATE ON REPRODUCTIVE TECHNOLOGIES FOR ENDANGERED CLOUDED LEOPARD

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Clouded leopards are endangered felids of Southeast Asia that the wild populations are declining due to habitat loss and hunting in range countries. The male aggression, high incidence of abnormal sperm and uncertain ovarian activity of females have been stated that limit the captive breeding success. Therefore, the development of optimal assisted reproductive technologies for this species is recommended. Over two decades, several assisted reproductive techniques have been applying in clouded leopard to overcome the limitation that effected on breeding success in both genders in term of reproductive biology, physiology and behavior. In male, semen collection and sperm preservation protocol have been optimized for genome resource banking and further assisted techniques applying. According to a high incidence of abnormal sperm in most captive males, sperm selection was attempted. In our recent study two sperm preparation methods included 1) simple washing or 2) the single layer centrifugation (SLC) method was applied prior to cryopreservation (n=12). The sperm motility, intact acrosome and sperm with normal tail in chilled and frozen-thawed semen samples were increased after processed by SLC. The heterologous IVF in the SLC-processed group had significantly higher fertilization rate than the simple washing group (P <0.05). In female clouded leopard, the fecal steroid hormone metabolites (i.e. estradiol, progesterone, glucocorticoid) have been studied for their reproductive physiology (estrous cycle, ovulation pattern and pregnancy). Exogenous gonadotropins administration were also attempted to stimulate ovarian function for assisted reproductive techniques such as artificial insemination (AI), in vitro fertilization (IVF) or others. Our current study, we have been attempting to optimize stimulating protocol for AI using eCG and pLH with promising results and less adverse effects for animals. To date, three reports of artificial insemination success has reported from fresh semen (Howard et al., 1996), chilled semen (Tipkantha et al., 2016) and frozen semen (Comizzoli et al., 2017). In conclusion, to select a good quality spermatozoa for further apply with optimal assisted techniques would be promising for propagation and sustainable the genetic variation of this endangered species in the future.