GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

Case Study

CONSERVATION OF EXOTIC AND ENDANGERED ANIMALS THROUGH BIOTECHNOLOGY

Sanny C. Babera[1] & Claro Mingala[2]
[1]Central Luzon State University, Science City of Munoz, Nueva Ecija 3120, Philippines
[2]Philippine Carabao Center-Gene Pool, Science City of Munoz, Nueva Ecija 3120, Philippines
*Corresponding author's e-mail: sannybabera@gmail.com

ABSTRACT
Maintaining the biodiversity of animals in the ecosystem could lessen the effect of environmental imbalance. Conservation and preservation of endangered animals using different methods in biotechnology could save important genetic materials for future reconstruction of extinct species or even the most endangered one. DNA fingerprinting is important in identifying traits vital to understand breeding peculiarities of wild animals specially birds. Genetic resource bank could facilitate the longer storage of diverse endangered and exotic genetic material.

KEY WORDS: DNA fingerprinting, artificial insemination, in-vitro fertilization, cryopreservation, wild animals.

INTRODUCTION

The continuous dwelling of man to expand agricultural land for livestock and crop production, housing community, new area for human habitat and industries will lessen the habitat of wild animals causing them to migrate into smaller and inhabited open space. The size of the land remains but as the population of man increases and for wild animals are decreasing area of habitat for both becomes limited. According to the International Union for the Conservation of Nature (IUCN) an animal or organism that is becoming extinct are termed endangered species (IUCN, 2010). The decreasing trend will continue if no appropriate action to maintain the degradation of wildlife population. Conservation and preservation of endangered animals is necessary to balance and maintain the functioning of ecosystem (Henson, 1992). Preservation of endangered species are in numerous methodology. One way is through biotechnology by reproductive molecular characterization, planned genetic breeding and by germ plasm preservation. This procedure is used successfully in domestic animals. However, it restricts the usage in wild animals due to various reasons. Despite the conservation of this animals into zoo's and protected reservation areas, gene banking of diverse genetic materials is also established to assure the protection of animal species from extinction in the ecosystem. The objective of this review is to lay down the methods used in successful conservation of endangered animals using biotechnology.

Mammal Biotechnology Conservation

Artificial insemination based on cattle procedure were adapted (Watson, 1978) and a pioneer method in reproductive biotechnology performed in the propagation of endangered animals (Wildt *et al*., 1995). The success of AI in various African wild ungulates is coupled with cryopreserved spermatozoa (Wildt *et al*., 1993, Bartels *et al*., 2001, Monfort, 2001, Pope, 2001, Rail, 2001, Solti *et al*., 2001) and in-vitro capacitation of sperm in *Oryx dammah* (Roth *et al*., 1998). Also, tested for larger wild animals like White Rhino (Hermes *et al*., 2008). The combination of AI and embryo transfer assures longer genetic variability (Holt and Pickard, 1999). In contrast for cheetah was not so successful (Wildt *et al.,* 2001). Knowledge on reproductive anatomy and phenomenon is needed like for Arabian oryx (Oryx leucoryx) have been studied (Wilson *et al*., 2006).

Cloning and stem cell in later years "are on debate" (Critser *et al*., 2003; Holt *et al*., 2004) for practical conservation. However, the procedure was successful in *Ovis orientalis musimon* using oocytes from *Ovis aries* (Pasquialino *et al*., 2001). However, the use of somatic cell nuclear transfer (SCNT) for the recovery of the specie has become successful (Folch *et al*., 2009). Therefore, SCNT can be used to regenerate previously extinct animal species. Offspring's from "baboon, rhesus macaque, marmoset, gorilla, Indian desert cat, ocelot, tiger, African wild cat, Armenian red sheep, water buffalo, gaur, red deer, llama and caracal" were produced from IVF and embryo transfer (Pukazhenthi and Wildt, 2004). However, IVF and embryo transfer for gene pool conservation are potential to use (Loskutoff *et al.,* 1995; Pope, 2000; Pukazhenthi and Wildt, 2004).

Despite studies are being done in wild animals, only AI is frequently performed (Pukazhenthi *et al*., 2006). The applications of biotechnology are selective for specific specie. The case of bucardo (*Capra pyrenaica pyrenaica*) declared extinct in year 2000 by the Spanish government but scientist were able to reconstruct via domestic animal as donor closely related to the specie. Saving an egg cell from deceased animal (Comizzoli *et al*., 2000) to freezing ovary and extract ovum from elephant, marmoset, wombat and wallaby has been possible (Lee *et al.,* 2004 and Paris *et al.,* 2004). Moreover, semen cryopreservation of endangered gazelles (*Gazelle gazelle, G. dorcas and G. gazelle acaiae*) in Israel was also successful (Saragusty *et*

al., 2006). The potential of this method in making the reproductive material from post mortem contribute to the effort to maintain biodiversity in the wild.

Biotechnology Approaches in Avians

According to Leirz *et al*. (2012) they have developed an improve semen collection for macaws, cockatoos, parrots and amazons for AI. The techniques that were developed are a breakthrough in the advancement for avian AI. They were able to collect semen of more than 100 species mostly from endangered birds. This development contributes to the collective effort of conservationist to fast tract the decreasing population of endangered birds. DNA fingerprinting had gained importance in understanding the mating behavior of birds (Burke and Bruford, 1987 and Wetton *et al*., 1987). The *Catharacta maccormicki* (south polar skua) (Millar *et al*., 1992), purple swamphen (*Porphyrio porphyrio*) found out that this are communal breeder (Lambert *et al*., 1994), the Australian superb fairywren (*Malurus cyaneus*) naturally has extra-pair copulations (Mulder *et al*., 1994). Furthermore, Gibbs *et al*. (1990) study the red-winged blackbirds (*Agelaius phoeniceus*) flight areas of breeding colony. It also determines the species hybrids (Ma *et al*., 1997). Moreover, from DNA fingerprinting of non-invasive determination of sex are develop (May *et al*., 1993 and Miyaki, 1992) and model of determining kin among animals (Hamilton, 1964). Owens *et al*. (1995) also have observed the behavior of Eurasian dotterel (*Charadrius morinellus*), "a species with sex-reversed plumage and polyandrous behavior". The establishment of the avian mating system, using minisatellite DNA fingerprinting was applied (Triggs *et al*., 1991). This is to deteremine population of birds and gene pool diversity (Triggs *et al*., 1992 and King *et al*., 2000), and was verified in blue duck (*Hymenolaimus malacorhynchos*). Moreover, determining genetic variables (Ardern *et al*., 1997) and "breeding behavior" (Ardern *et al*., 1997) in the highly threatened black robin (*Petroica traversi*) on New Zealand's Chatham Islands are proved. The tool were also used in other animals including fish in evaluating the population diversity of California Channel Island fox (*Urocyon littoralis*) (Gilbert, 1990) and the humpback whale (*Megaptera novaeangliae*) (Baker *et al*., 1993). In addition, minisatellite procedure was tried to map specific trait of other specie (Hanotte *et al*., 1992 and Hermans *et al*., 1990).

Gene bank Facility

The establishment of gene bank facility can store diverse genetic materials of exotic and endangered animals. The efficiency of conservation for the genetic analysis of specific biotechnology tool must require. This facility will help conservationist all around the world to exchange and share resources effectively (Pizzi *et al*., 2013).

Limitations of using biotechnology for endangered animals

Studies on farm animals using artificial insemination has been commercially used however, less information on reproductive function for wild animals (Comizzoli, 2009). Concurrent to this, commercially produced hormones are ineffective when used to wild animals to induce ovulation (Scheiwe *et al*., 1991) and the effect of indefinite follicular development (Thompson and Monfort, 1999). In AI, inducing ovulation ensures high rate of conception. Age and social acts affect the viability of gametes in deer (Jabbour *et al*., 1997). Some species like deer has social hierarchy that only dominant males are producing active sperm cells. Confined rearing of wild animals hinders behavioral instinct like aggressiveness in courting. Furthermore, the condition of wild animals while on restricted space commonly affects their state of health and reproduction (Lasley *et al*., 1994). Scarce studies on wild animal biotechnology causing failure to create a model protocol for specific adaptation of assisted reproductive technology (Comizzoli, 2000). Moreover, limited source of viable gametes from various wild species for in depth experiment (Leibo and Songsasen, 2002).

CONCLUSION

The use of artificial insemination is practical and effective method to repopulate the endangered mammal and bird species. The method has become widely used when backed by gamete cryopreservation. However, thorough knowledge on the systems of reproduction of the different endangered species is required. DNA fingerprinting has established breeding system of birds and able to tract the pedigree and ensure the diversity of genetic pool in the environment. The establishment of gene bank ensures the availability of genetic material in reconstructing highly endangered animals to bring back in the ecosystem.

REFERENCES

Ardern, S.L., Lambert, D. M., Rodrigo, A. G. and Mclean, I.G. (1997) The effects of population bottlenecks on multilocus DNA variation in robins. *J Hered.* 88:179-186.

Ardern, S.L., Ma, W., Ewen, J.G., Armstrong, D. P. and Lambert, D. M. (1997) Social and sexual monogamy in translocated New Zealand robin populations detected using minisatellite DNA. *Auk*, 114:120-126.

Baker, C.S., Gilbert, D. A., Weinrich, M.T., Lambertsen, R., Calambokidis, J., McArdle, B., Chambers, G. K. and O'Brien, S. J. (1993) Population characteristics of DNA fingerprints in humpback whales (*Megaptera novaeangliae*). *J Hered*, 84:281-290.

Bartels, P., Friedmann, Y., Lubbe, K., Mortimer, D., Rasmussen, L. A. and Godke, R. A. (2001) The live birth of an eland (Taurotragus oryx) calf following estrous synchronization and artificial insemination using frozen thawed epididymal sperm. Theriogenology 2001; 55 :38 1 abstr.

Burke, T. and Bruford, M. (1987) DNA fingerprinting in birds. Nature 327:149-152.

Comizzoli, P., Mermillod, P. and Mauget, R. (2000) Reproductive biotechnologies for endangered mammalian species. Reproduction Nutrition Development, 40, 493–504.

Comozzoli, P., A. E. Crosier, N. Songsasen, M. S. Gunther, J. G. Howard and D. E. Wildt (2009) Advances in reproductive science for wild carnivore conservation. Reprod Dom Anim 44 (Suppl. 2), 47-52; doi: 10.1111/j.1439-0531.2009.01374.x

Critser, J.K., Riley, L.K., and R.S. Prather (2003) Application of nuclear transfer technology to wildlife species. In "Reproductive Science and Integrated Conservation" (Eds W. V. Holt, A. R. Pickard, J. C. Roger and D. E. Wildt.) pp.195-208. (Cambridge University Press: Cambridge, UK.)

Folch, J., Cocero, M.J., Chesné, P., Alabart, J.L., Domínguez, V., Cognié, Y., Roche, A., Fernández-Arias, A., Martí, J.L., Sánchez, P., Echegoyen, E., Beckers, J.F., Sánchez, A., Bonastre and Vignon, X. (2009) First birth of an animal from an extinct subspecies (*Capra pyrenaica pyrenaica*) by cloning. Theriogenology, 71, 1026-1034.

Gibbs H. L., Patrick, J., Weatherhead, P.J., Boag, P.T., White, B.N., Tabak, L.M. and Hoysak D.J. (1990) Realized reproductive success of polygynous red-winged blackbirds revealed by DNA markers. *Science*, 250:1394-1397.

Gilbert, D.A., Lehman, N., O'Brien, S.J. and Wayne, R.K. (1990) Genetic fingerprinting reveals population structure in the California Channel Islands fox. *Nature*, 344:764-767.

Hamilton, W.D. (1964) The genetical evolution of social behaviour, I and II. *J Theor Biol* 1964, 7:1-52.

Hanotte, O., Bruford, M.W. & Burke, T. (1992) Multilocus DNA fingerprints in gallinaceous birds: General approach and problems. *Hered*, 68:481-494.

Henson, E.L. (1992) In situ conservation of livestock and poultry, FAO Animal Production and Health Paper 99 112p.

Hermans, I., Morris, F.C.A., Chambers, G.K., Towers, N.R. & Jordan T.W. (1990) Assessment of DNA fingerprinting for determining genetic diversity in sheep populations. *Animal Genet*, 24:385-388.

Hermes, R., Goritsz, F., Saragutsy, J., Sos, E., Molnar, V., Reid, C.E., Schwarzenberger, F. and Hilderbrandt, T.B. (2008) First Successful artificial Insemination with Frozen Thawed Semen in Rhinoceros. Theriogenology: An International Journal of Animal Reproduction, DOI: 10.1016/ j.theriogenology.10.008.

Holt, W.V. & Pickard A.R. (1999) Role of reproductive technologies and genetic resource banks in animal conservation. Reviews of Reproduction, 4, 143-150.

Holt, W. V., Pickard A.R. and Prather, R.S. (2004) Wildlife Conservation and Reproductive Cloning. Reproduction 127, 317-324. Doi: 10.1530/REP.1.00074

IUCN (2010) IUCN Red list of Threatened species. Version 2010.4. Available at: www.iucnredlist.org accessed 01 June 2011.

Jabbour, H.N., Hayssen, V. & Bruford, W. (1997) Conservation of deer: contributions from molecular biology, evolutionary ecology, and reproductive physiology, J. Zool. Lond. 243 461–484.

King, T. M., Williams, M. and Lambert, D. M. (2000) Dams, ducks and DNA: identifying the effects of a hydro-electric scheme on New Zealand's endangered blue duck. *Conserv Genet*, 1:103-113.

Lambert, D.M., Millar, C.D., Jack, K., Anderson, S. and Craig, J.L. (1994) Single- and multilocus DNA fingerprinting of communally breeding pukeko: Do copulations or dominance ensure reproductive success? *Proc Natl Acad Sci USA*, **91:**9641-9645.

Lasley, B.L., Loskutoff, N.M. & Anderson, G.B. (1994) The Limitation of conventional breeding programs and the need and promise of assisted reproduction in non domestic species. Theriogenology 41 119-132.

Lee, D. M., Yeoman, R.R., Battaglia, D.E., Stouffer, R.L., Zelinski-Wooten, M.B., Fanton, X.W. & Wolf D.R. (2004) Live birth after ovarian tissue transplant. *Nature* **428,** 137-138. doi:10.1038/428137A

Leibo, S.P. and Songsasen, N. (2002) Cryopreservation of gametes and embryos of non-domestic species. *Theriogenology* 57, 303-326. doi: 10.1016/ S0093-691X(01) 00673-2.

Leirz, M., Reinschmidt, M., Muller, H. and Neumann, D. (2012) A novel method for collection and artificial insemination in large parrots (Psittaciformes). Doi: 10.10338/ srep02066.

Loskutoff, N.M., Bartels, P., Meintjes, M., Godke R.A. & Schiewe, M.C. (1995) Assisted reproductive technology in nondomestic ungulates: a model approach to preserving and managing genetic diversity. *Theriogenology* 43, 3-12. doi:10.1016/ 0093-691X(94)00005-F

Ma, W. & Lambert D.M. (1997) Minisatellite DNA markers reveal hybridization between the endangered black robin and tomtits. *Electrophoresis*, 18:1682-1687.

May, C.A., Wetton J.H. & Parkin D.T. (1993) Polymorphic sex-specific sequences in birds of prey. *Proc Roy Soc Lond B*, 253:271-276.

Millar, C. D., Lambert, D. M., Bellamy, A. R., Stapleton, P.M. & Young E.C. (1992) Sex-specific restriction fragments and sex rations revealed by DNA fingerprinting in the brown skua. J Hered. 83:350-355.

Miyaki, C.Y., Hanotte, O., Wajntal, A. and Burke, T. (1992) Sex-typing of Aratinga parrots using the human mini-satellite probe 33.15. *Nucl Acids Res*, 20:5235-5236.

Monfort, S.L. (2001) Cervid reproductive biology and ART. Proc 1st Int Symp Assisted Reprod Technol Conservation Genet Manage Wildlife, Omaha's Henry Doorly Zoo, 2001;5 l-55.

Mulder, R.A., Dunn, P.O., Cockburn, A., Lazenby-Cohen K. A. and Howell, M.J. (1994) Helpers liberate female fairy-wrens from constraints on extra-pair mate choice. *Proc Roy Soc Lond B*, 255:223-229.

Owens, I.P.F, Dixon, A., Burke, T. and. Thompson, D.B.A (1995) Strategic paternity assurance in sex-role reversered Eurasian dotterel (*Charadrius morinellus*): Behavioral and genetic evidence. *Behav Ecol*, 6:14-21.

Paris, M.C., Snow, M., Cox, S.L. and Shaw, X.M. (2004) Xenotransplantation: a tool for reproductive biology and

animal conservation. *Theriogenology* 61, 277-291. doi: 10.1016/ S0093-691X(03) 00234-6

Pasqualino, L., Ptak, G., Barboni, B., Fulka, J., Cappai, P. and Clinton, M. (2001) Genetic rescue of an endangered mammal by cross-species nuclear transfer using post-mortem somatic cells. Nature Biotechnology 19, 962-964. Doi: 10.1038/ nbt1001-962.

Pizzi, F., Caroliz, A.M., Landini, M., Gallucio, N., Mezzelani, A. and Melanesi, L. (2013) Conservation of endangered animals: From biotechnologies to digital preservation. Natural Science. Vol 5, No. 8, 903-913.http:// dx.doi.org/10.4236.ns.2013.58109

Pope, C.E. (2001) Antelope reproductive biology and ART. Proc 1st Int Symp Assisted Reprod Technol Genet Manage Wildlife, Omaha's Henry Doorly Zoo, 2001;45-55.

Pope, C. E. (2000) Embryo technology in conservation efforts for endangered felids. *Theriogenology* 53,163-174. doi: 10.1016/S0093-691X(99)00249-6.

Pope, C. E. (2000) Embryo technology in conservation efforts for endangered felids. *Theriogenology* 53,163-174. doi: 10.1016/S0093-691X(99)00249-6

Pukazhenthi, B.S., & Wildt, D.E. (2004) Which reproductive technologies are most relevant to studying, managing and conserving wildlife? Reprod. fertil. Dev. 16, 33-46. Doi: 10.1071/RD03076

Pukazhenthi, B.S. & Wildt, D.E. (2004) which reproductive technologies are most relavant to studying, managing and conserving wildlife? *Reprod. Fértil. Dev.* 16, 33-46. doi:10.1071/ RD03076

Pukazhenthi, B.S., Comizzoli, P., Travis A.J. & Wildt, D.E. (2006) Applications of emerging technologies to the study and conservation of threatened and endangered species. In "Reproduction, Fertility and Development.18,77-90. Doi: 10.1071/RD05117

Rail, W.F. (2001) Cryopreservation of mammalian embryos, gametes and ovarian tissues: current issues and progress. In: Wolf, DP and Zelinski-Wooten, M (eds.), Assisted Fertilization and Nuclear Transfer in Mammals. Totawa, NJ: Humana Press, 2001; 173-187.

Roth, T.L., Weiss, R. B., Buff, J. L., Bush, L. B., Wildt, D.E. and Bush, M. (1998) Heterologous in vitro fertilization and sperm capacitation in an endangered African antelope, the scimitar-horned oryx (*Oryx dammah*), Biol. Reprod. 58 475–482.

Saragusty, J., Gacitua, H., King, R. and Arav, A. (2006) *Post-mortem* semen cryopreservation and characterization in two different endangered gazelle species (*Gazella gazella* and *Gazella dorcas*) and one subspecies (*Gazella gazelle acaiae*). Theriogenology, 66, 775-784.

Schiewe, M.C., Bush, M., Phillips, L.G., Citino, S. and Wildt D.E. (1991) Comparative aspects of estrus synchronization, ovulation induction, and embryo cryopreservation in the scimitar-horned oryx, bongo, eland, and greater kudu, J. Exp. Zool. 258 75–88.

Solti, L., Crichton, E.G., Loskutoff, N.M. and Cseh, S. (2001) Economical and ecological importance of indigenous livestock and the application of assisted reproduction to their preservation. Theriogenology 2001; 53:149-162.

Thompson, K.V. and Monfort, S.L. (1999) Synchronisation of oestrous cycles in sable antelope, Anim. Reprod. Sci. 57 185–197.

Triggs, S. J., Williams, M., Marshall, S.J. and Chambers, G.K. (1991) Genetic relationships within a population of blue duck (*Hymenolaimus malacorhynchos*). *Wildfowl*, 42:87-93.

Triggs, S.J., Williams, M.J., Marshall, S.J. and Chambers, G.K. (1992) Genetic structure of blue duck (*Hymenolaimus malacorhynchos*) populations revealed by DNA finger printing. *Auk*, 109:80-89.

Watson, P.F. (1978) Artificial Breeding of Non-Domestic Animals. Zool. Soc. Lond. Symp. 43. Academic Press, London.

Wetton, J.H., Carter, R.E., Parkin, D.T. and Walters, D. (1987) Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature*, 327:147-149.

Wildt, D.E., Seal, U.S. and Rall W.F. (1993) Genetic resource bank and reproductive technology for wildlife conservation. In: Cloud JG, Thorgaard GH (eds.), Genetic Conservation of Salmonid Fishes. New York: Plenum Press, 1993;159-173.

Wildt, D.E., Pukazhenthi, B.S., Brown, J.L., Monfort, S., Howard, J.G. & Roth, T.L. (1995) Spermatology for understanding, managing and conserving rare species. Reprod. Fertil. Dev. 7:811–824.

Wildt, D.E., Ellis, E. & Howard X.G. (2001) Linkage of reproductive sciences: from 'quick fix' to 'integrated' conservation. J. Reprod. Fértil. Suppl. 57, 295-307.

Wilson, R.D., Fricke, P. M., Leibfried-Rutledge, M. L., Rutledge, J. J., Panfield C. M. & Weigel, K.A. (2006) In Vitro Production of Bovine Embryos using ex-sorted sperm. Theriogenology 65 pp. 1007-1015.