Possible Application of Animal Reproductive Researches to the Restoration of Endangered and/or Extinct Wild Animals - Review -

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ABSTRACT : As described here, most recently developed methods for improving reproduction performance of domesticated animals such as cattle, swine and chicken have been considered to be also usable for restoring some sorts of endangered and/or extinct wild animals in the very near future. Especially, the techniques for *in vitro* storage of gametes obtained from dead animals shortly after the death, probably 24 h following the sacrifice are also available for obtaining some of experimental specimens. In case of the endangered animals, nobody will be allowed to use any tissues from the living animals, therefore, e.g., the use of skin tissues from these bodies is another possibility of restoring the living animals. Regarding the use of skin tissues, the most highly usable tools must be the cloning techniques for reviving rare cells from the living body. Most possible techniques for cloning cells is nuclear transfer from rare species to highly relative species, and this is the case of germ cells, e.g., primordial germ cells (PGCs) of avian species. One of the possibilities is the nuclear transfer of Crested Ibis (*Nipponia nippon*) to the PGCs of chicken, resulting in the PGCs with transferred nucleus from the ibis. In mammalian species, the same procedure as in the case of birds would be successful, e.g., the removed nucleus from Giant Pandas will be transferred to the cell, such as somatic cells or germ cells from black bears or lesser pandas, leading to the production of transnucleared cells in the body of female black bears. These two cases are most promising techniques for reviving endangered animals in the world, particularly in Asian countries, mainly in China. As a coclusion, possible production of cloned animals carrying transnucleared cells from endangered animals, such as Giant Pandas and Crested Ibis, may be reproduced gradually in the near future. Scientists are, therefore, required to convert the paradigm from domestic animals to wild animals, icluding endangered and/or extinct animals on the earth. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 7 : 1026-1034*)

Key Words : Genetic Erosion, Genetic Diversity, Biodiversity, Germ Cells, Review

INTRODUCTION

The biological diversity will be defined as the millions of plants, animals, and microorganisms, the genes they contain, and the intricate ecosystems they help build into the living environment (Dobson, 1998; Primack, 1995, 1998). By this definition, biological diversity must be considered on three levels: species diversity, genetic diversity and community diversity (Higuchi, 1996; Kawabata, 1998; Klingholz, 1998; Obara, 1998; Obara and Hotta, 1998). All these levels of biological diversity are necessary for the continuted survival of species and natural communities, and all are also important to people (Ishijima et al., 1997; Long, 1998). Species diversity represents the entire range of evolutionary and ecological adaptations of species to particular environments (Washitani, 1999; Williams, 1999). The diversity of species provides people with resource and resource alternatives. Genetic diversity is necessary for any species to maintain reproductive vitality, resistance to diesease, and the ability to adapt to changing conditions. Genetic diversity in domestic plants and animals is of particular value in the breeding programs necessary to sustain and improve modern agricultural species. Community-level diversity represents the collective response of species to different environmental conditions (Dobson, 1998).

This concept about biodiversity was recently spread out at a strech on a worldwide scale, creating new field of science such as conservation biology (Anderson and Bodo, 1992). This idea invaded the researches on animal reproduction and/or breeding, focusing mainly on the application of newly developed biotechnology to the research field of wild animal sciences (Fujihara, 1999a, b, c). In this sense, significant application of various biotechnological tools to the possible rescue of endangered and/or extinct animals on the earth should be considered heart and soul (Fujihara and Xi, 1999a, b). With reference to conservation biology, animal genetic resources have been considered to be very important things for producing some of beneficial commodities as foods for human being (Hackett et al., 1998; Shiva, 1997; Tanabe et al., 1996).

Recent researches regarding the field of reproductive physiology of wild animals showed that

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reproductive function of male wild animals seemed to be remarkably declined, though there are no clear reason for this very serious problem (Fang et al., 1998a, b; Fan et al., 1999a, b, 2000). Typical specimens for this question are probably the case of Giant Pandas in China and Crested Ibis in Japan and these two animals, mammals and birds, are very popular and famous in the world, especially in Asia (Fang et al., 1997a, b, c; Schaller et al., 1985). The latter bird has also become of major interest lately due to the hatch of new baby Crested Ibis in Sado, Japan (Xi and Fujihara, 1999, 2000).

Of these two most important and endangered animals, the Giant Pandas have been examined for eveything including population sizes, reproductive performances, failure of natural mating for both male and female animals, declined fertility, lower percentage of delivery, false parturition behavior, lower growth rate of baby animals and many other things related to the decrease of population size of this special animal (Fang et al., 1997d, e, f). Almost all of the research results originated from the Research Base for Giant Pandas in Chengdu, Sichuan, China (Feng et al., 1991a, b, 1994). Furthermore, the results of some basic researches about this animal have been reported in China, though most of them were written in Chinese, making these valuable fruits ruinous forever (Feng et al., 1996, 1997).

On the one hand, with reference to the Crested Ibis, there are also a lot of references written by Chinese scientsits who have been mainly working at the Feeding and Breeding Center for Crested Ibis, Yangxian, Shaanxi Province, China (Xi et al., 1995, 1997, 1999a). Some of the facilities for researches on Crested Ibis in this center are still in insufficient, because main investigations for this center would be focused on feeding and breeding of this bird, particularly from basical researches (Xi et al., 1999b; Xi and Fujihara, 1999). As compared with the case of Giant Pandas (around 800 - 1000 heads) (Zhang and He, 1998; Nakaya and Fujihara, 2000), the Crested Ibis is in the situation of remarkably reduced number of birds, being approximately only less than 200 birds at the present moment (Xi and Fujihara, 2000).

Almost two decades ago, some of the researches have been carried out about Crested Ibis (*Nipponia nippon*) in Sado, Japan, without obtaining nothing as a results (Ishii, 1994; Fujihara, 1996). As reported by all masscommunication in Japan, a happening of the century occurred in Sado, Japan, reporting the birth (hatching) of new baby Crested Ibis (Kunimatsu, 1999; Liu et al., 1995, 1997). This big news gave us, all of the Japanese people, some new ideas for protecting endangered animals in our country, including young and old, male and female in Japan (Xi and Fujihara, 2000a, b). This also mean the awakening of animal protection and environmental protection on the earth all over the country, though this is too late for us to restore these critical animals in the world (Fujihara, 1999).

On the one hand, a lot of animals other than the Crested Ibis in Sado are still in a critical situation, though no special attention was paid to these animals due probably to indifference to such sort of things by general people in this country (Obara, 1998).

This review, therefore, will deal with most recently developing items, focusing on the citations from only the books and/or reviews, not from individual papers appeared in the Journals, including the reports published mainly in Asian countries, judging from the author's own responsibility and prejudice.

CRISIS OF GENETIC EROSION

The problems regarding genetic erosion were spread out all over the world, including plant and animal kingdoms, leading to the present status of living things on the earth (Cadbury, 1997; Colborn et al., 1997). For millions of years, all kinds of living things on the earth must have had to change their several characteristics so as to make suitable adaptation to the respective areas on the earth (Long, 1998). As the results, a lot of varieties of animals and plants have been evolved in many areas on the earth, forming biodiversities with their respective parts of the world (Tanabe et al., 1996).

However, even if these theoretical evolution of animals has been proceeded for millions of years, the development of the life of human being induced some extraordinal changes at any part of the earth, which were probably developed to improve our human daily life, including foods, clothes, transportation and the very closely related things for us (Reiss and Straughan, 1996). These changeable things continued to improve our life style, particularly every countries which will belong to the developed countries categorized according to the classification of living standards (Primack, 1995, 1998). The demands of these people living in these developed countrie to make much more improvement in every ways, in no connection with any other living things (Primack, 1995, 1998). Thses earth environment destructive actions by human being brought about some irrecoverable damage to even our human daily life, resulting in the genetic erosion and the loss of gene diversities (Primack, 1995, 1998).

In the present status of the earth environments is probably contrary to the former ones compared with some ages during around five decades, a half century. In particular, the economical conditions of the countries which belong to Western areas in the world gave us a huge number of troubles regarding the earth

environments (Fujihara, 1999b, c). In this respect, extremely highly developed industries were main source for the earth environmental pollution, though these also enhanced the level of human life style, including cars, TV, cameras, watches, radios, cassettes and some other mechanical things closely related to our daily life (Fujihara, 1996, 1997). These newly renovated things are surely closely linked with our life style, so that even if these things were completely removed from our daily life, all the people probalby go to some kinds of panic condiations without fail.

Therefore, even when the earth were heavily polluted and biodiversities were seriously damaged, the life styles of human being will never change in the future, though this is very importan thing for the renewal of the earth (Fujihara, 1996, 1997). This suggest that the continuation of these illegal transformation of natural things by human being probably bring about the end of earth in the very near future (Fujihara, 1999c). In this sense, the sciences of biodiversities, genetic resource conservation and the prevention of genetic erosion may be of fundamental importance for future life of living things on the earth (Fujihara, 1997).

Recent research trends for wild animals extaordinally focused on the biodiversity in the world, including genetic erosion, genetic diversity and animal diversity, resulting in the tendency to create some kinds of new types of animals, for both wild and domesticated ones (Fujihara, 1999c; Fujihara and Xi, 1999). This tendency of researches was also directed toward the field of animal reproduction sciences, leading to the application of researches to the restoration of extinct and/or endangered animals, including domesticated and wild livings (Anderson and Bodo, 1992).

The present review will, thus, deal with some of the information whether basical researches involving animal reproductive physiology may have some responsibility for the restoration of extinct and/or endangered animals in the world. However, main point will be focused on the reproductive researches from avian species, including exceptionally some sorts of mammals.

PRESENT SITUATIONS OF ENDANGERED AND/OR EXTINCT ANIMALS

If we will pay a special attention to the area of Asian countries, a couple of animals, including mammals and birds, would be considered as endangered animals (Xi and Fujihara, 2000a, b). Giant Pandas and Crested Ibis might, for example, be taken notice of, though some other animals might be surely included in this category (Shaller et al., 1985; Feng and Fu, 1994). However, one of them is probably a rare bird ibis (*Npponia nippon*) which was already reported excessively throughout Japan, being hatched in Sado, Niigata Prefecture (Xi and Fujihara, 2000a, b).

This case would be an example of the present conditions of wild animals which were gradually squeezed through long decades by highly and economically developed human lives on the earth. The present characteristics of earth are generally said to be in very critical conditions which may indicate the end of this planet. A lot of wild animals are vanishing from the earth, along with their entire ecosystem, suggesting the possibility of abnormal changing of the earth including farm lands which were enriched by some kinds of wild animals (Long, 1998).

The main reason for these endangered animal kingdom would be brought about by some of excessively used chemical materials for our human lives, especially during the past five decades and more. Most of them were used mianly for improving agricultural production, animal production, house keeping and the related things. All of these chemicals were almost harmful to living things, needless to say, including wild and domesticated animals, resulting in drastically decreased number of animal species on the earth (Cadbury, 1997; Colborn et al., 1997).

Most recently, these chemicals were shown to be the materials called at first "Environmental Hormones", but this was lately newly named as "Endocrine Disturbing Chemicals". These chemicals are shown to have some incredibly serious damages to all of human kinds, including male and female. The readers should refer to a world-shaking book entitled "Our Stolen Future" written by Colborn (1997) which has been said to be the book next to the book named "Silent Spring" by Carson (1982). The later book predicted the present situations of the earth even more than a half century ago, and the former one gave us very strongly shrewd warning against the present severe conditions on this planet.

Judging from these various circumstances, some unconsious losses of wild animals would be observed in everywhere in the world, unless we have to make a resolution to change our paradigm in the very near future in the world. This has been considered to be probably most important and urgent things for human beings. In the present times, most of the scientists and/or some other related peoples in the world are aware of danger of earth, vanishing all of the living things from the earth (Obara, 1998; Obara and Hotta, 1998).

Lately, some of the zoos in the world tend to participate in the preservation of endangered and/or extinct animals, using recently developed various biotechnologies (Kawabata, 1998). They also aim to have some responsibility for genetic erosin or collapse of biological diversity on the earth, employing genetic

engineering and some of biotechnology (Tanabe et al., 1996).

To our most surprising, a magnificient project has been proposed by some scientists in Japan and Russia in which sperm nucleus (DNA) obtainable from frozen testis of mammoth in Siberia would be used for microinjection into the ova derived from the ovary of Asian or African elephants (Goto, 1998a, b, c, d). If this long-ranged and great scientific project would be successful and realized in Japan and/or Russia, we may also have to make some complete change our paradigm. In addition to this, we also must extend our past and old conceptions to the ides that human being have to live together with any kind of animals and plants on the earth from now on.

RECENT TRENDS IN RESEARCHES ON MAMMALIAN REPRODUCTION

In this section, the author would refer mainly to basical researches which will be much more applicable to the retoration of endangered and/or extant wild animals, especially considering the animals in the areas of Asian countries. Therefore, the author might have paid special attention to the researches which have successfully been carried out in his laboratory, including mainly three directions: (1) establishment of *in vitro* culture system for bovine oocytes derived from the ovary obtained by some of local slaughterhouses (Zhao et al., 1998b; Ohboshi, 1999). This tequniche would be effectively utilizable for *in vitro* development of immature oocytes from died animals and/or endangered ones in wild conditions. One of the papers, which was published in the Internaitonal Journals, attracted a special attention which showed a possibility of using Japanese Kampo Medicines (JKMs) for *in vitro* culture of bovine oocytes in place of fetal calf serum (FCS) or calf serum (CS) and in vitro fertilization of cultured ova (Zhao et al., 1997, 1998a). These JKMs may refer to some sorts of most traditional oriental or Chinese medicines which have already been reported for human reproduction as one of the promising materials, focusing mainly on the treatment of infertility in women (Hagino, 1996a, b; Koyama et al., 1988; Sato, 1998). If these JKMs have some unique functions similar to some other materials such as fetal calf serum (FCS), calf serum (CS) and bovine serum albumine (BSA), much more efficient production of *in vitro* derived gametes will be realized in the future (Zhao et al., 1998).

On the one hand, recently, it has been shown that some of the skin cells taken out from wild animals such as the lesser pandas were sucessfully cultured outside the body, even if they were obtained after 24 h of death in the wild (Nakaya and Fujihara, 1999). This also suggests the possibility of utilizing of several tissues from dead animals for *in vitro* culture or preservation even when we might have a chance to obtain some materials from wild animals. This may also extends the field of researches of extant animals in wild conditions. Therefore, this kind of basic researches would be much more needed for future study on the protection and conservation of wild animals. (2) Possible establishment of *in vitro* preservation of primary oocytes derived from the ovary in local abattoirs. If this method would be successful in the future, some great progress may be shown in the preservation of unfertilized ova obtained from the ovary immediately after the death of wild animals (Otoi et al., 1996; Zhao et al., 1996; Ohoboshi, 1999). (3) *In vitro* preservation of gametes, spermatozoa and ova, would also be most important factors for the restoration of endangered and/or extant animals. There are some other excellent reviews concerning the gamete storage outside the body, inclusing non-domesticated animals (Fujihara, 1996; Watson, 1978; Wilmut et al., 1998). As for this purpose, considerable efforts have been made in order to restore one of the endangered Crested Ibis (*Nipponia nippon*) in Sado, Japan, though nothing has been obtained from this project (Ishii et al., 1994). Therefore, this method of gamete preservation outside the body would become most applicable for preservation of germ plasma from wild animals (Bakst, 1990; Crawford, 1990; Fujihara, 1994, 1996; Delany and Pisent, 1998; Lukaszewicz et al., 2000).

RECENT ADVANCES IN RESEARCHES FOR AVIAN REPRODUCTION

As for this section, the author would mainly refer to some basical experiments which have been carried out in his laboratory, due probably to the reason why several experimental results were obtained for the purpose of restoration of endangere and/or extant wild animals.

First of all, in this section, the main points are probably the production of transgenic chickens, *in vitro* transfer of foreign genes into chicken embryos, establishment of in vitro culture of primordial germ cells (PGCs), production of chimeric chickens by transferring germ cells and the likes (Aanderson, 1998; Ebara and Fujihara, 1999a, b; Eguma et al., 1999; Fujihara, 1999a). Another important point will be animal cloning, including nuclear transfer from the cells derived from endangered and/or extinct animals, being already trying in some of these kinds of animals in the world (Romanov et al., 1996; Pinkert and Murray, 1998; Fujihara, 1999b). For this purpose, nuclear transfer of avian primordial germ cells (PGCs), being very difficult in the present time due probably

to the hardness of cell membranes of PGCs (Furuta and Fujihara, 1999; Furuta et al., 1999; Inada et al., 1997, 1999). This germ cell is entirely different in several aspects from some other somatic cells, and PGCs are destined to form gonads which are also for producing germline transfer of exogenous genes (Farrell, 1995; Petitte et al., 1998; Furuta and Fujihara, 1999; Furuta et al., 1999).

Based on these complexed experimental results, most useful basic researches may be the cases which have been carried out in the field of animal cloning and the establishment of germ cell lines derived from embryonic germ cells (EGCs) and/or embryonic stem cells (ESCs) (Kuwana, 1993; Naito et al., 1994; Ives and Bedford, 1998; Zirkin, 1998; Yang and Fujihra, 1999a, b). The former method still seems very difficult to make a breakthrough by which avian cloning may be brought to realization in the future. On the one hand, the latter may be successfully carried out before long, leading to the results that some birds with germline chimeras will appear on the commercial bases (Wong et al., 1998).

Apart from some other important reseraches regarding avian reproductive physiology, most promising research works for producing cloned birds or chimeric chicken may require the techniques of gene transfer and sex reversal which will bring about great change of paradigm from the present situations (Abinawanto, 1997; Kagami and Hanada, 1997; Kagami et al., 1997).

Most recent results obtained in the author's laboratory suggest the possibility of chicken sperm vectors for transferring exogenous genes into avian developing embryos (Hasebe et al., 1998; Squires, 1998; Arima et al., 2000). His finding has been considered to become most effective tools for producing transgenic chicken via germ lines. On the one hand, in chicken embyos, exogenously introduced genes were successfully transferred to next generations via germ lines, producing offsprings carrying foreign genes (Ebara and Fujihara, 1999a, b; Furuta et al., 1999a, b: Furuta and Fujihara, 1999). These prospective experimental results may indicate that some of these techiniques will serve sufficiently.

POSSIBLE APPLICATION OF BASIC RESEARHCES TO RESTITUTION OF WILD ANIMALS

As mentioned above, fundamental researches regarding animal reproduction sciences are certainly applicable in many ways to the restoration and/or reconstitution of extinct or endangered animals on the earth (Fujihara, 1999b). For example, if *in vitro* culture of skin tissues from wild animals could be successful, a tiny amount of tissues from living endangered and/or extinct animals would be enough for the propagation of genes (DNA) to preserve outside the body for a long term. Therefore, the establishment of *in vitro* culture methods of skin tissues may bring about a significant breakthrough for the field of *in vitro* preservation of wild animal tissues. In the author's laboraotry, several trials has been done on *in vitro* culture of several tissues form various animals so as to develop much more easy techniques for preserving living cells outside the body. This method will be successfully applicable for *in vitro* preservation of the cells from wild life even when accidential sacrifice of the adult animals happened somewhere in the world (Nakaya and Fujihara, 1999).

FUTURE ASPECTS

The conservation of wildlife relates to the maintenance of living creatures which arouse popular sympathy and support at the present times. Only recently mankind recognized the importance of the genetic resources of farm animals embodied in the value of rare old domestic breeds. Thus, the professional preservation of domestic animals is a relatively young activity. Fortunately, animal breeders were able to preserve some valuable old populations, and in the last decades, however, the increasing popularity of this idea can be observed everywhere. The number of breeders recognizing the importance of non-commercial breeds, and willing to sacrifice profit for the sake of conservation, is also increasing. Lately, some of the countries in the world are aware of the genetic conservation of domestic livestocks in the world in order to form a global organization for rare breeds.

To prevent the genetic erosion from commercial breeds of livestock, modern methods of biotechnology are at present used in preservation programms, including molecular genetic screening, animal germplasma conservation strategy, embryo banks together with animal gene banks.

Although these above-mentioned matters have probably been focused on the reproduction of domesticated animals, and some of them are also applicable for restoring endangered and/or extinct animals. In this connection, special attention will be paid for animal cloning, nuclear transfer, *in vitro* preservation of gametes, including sperm cells, ova and embryos, cryopreservation of germ cells and the relates. The cryopreservation, in particular, vitrification method is presumably one of the most promising techniques for storing animal genetic resources in the future. This also implies that in the case of wild animals, especially when they have to be treated just immediately after the accidents or death so as to

obtain as many experimental samples as possible. To do this much more effectively, fundamental researches regarding tissue culture and nuclear transfer from wild animals to domesticated ones must have been established in the very near future. Thereafter, cloned animal cells would be preserved in liquid nitrogen (-196℃) until its reuse for restoring extinct animals.

Another important thing is the establishment of ES (embryonic stem) cell lines derived from embryonic cells or EG (embryonic germ) cell lines derived from germ cells at the early stage of embryonic development. However, this kind of experiment may need much more theoretical understanding before going to the trial with blind task.

CONCLUSION REMARKS

In conclusions, successful applications of basical research works being done using domestic livestocks to the restoration of endangered and/or extinct animals were extraordinally required by wildlife researchers in the world. However, an important but very difficult hurdle to be overcame before going to start the experiments will be needed. At the present time, most usable tools for reviving endangered animals are probably the technique of nuclear transfer or animal cloning using somatic cellls as nulcear donors. In this case, removing nucleus from donor cells will require highly sophisticated techniques before going to cell fusion with recipient cells from normal animal cells such as very closely related species cells.

Based on these materials, most promising tools for restoring endangered animals are probably animal cloning techniques, including somatic cells as donor's ones and germ cells as recipient cells from non-extinct species.

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REFERENCES

Abinawanto, K. Shimada and N. Saito. 1997. Sex-reversal effects of non-steroidal aromatase inhibition on aromatase (P450 arom) mRNA expression in adult chicken gonads. Jpn. Poult. Sci. 34:158-168.

Anderson, L. 1990. Genetic Conservation of Domestic Livestock. CAB Int., Vol. 1, London. pp. 78-86.

Alderson, L. and I. Bodo. 1992. Genetic Conservation of Domestic Livestock. CAB Int., Vol. 2, London. pp. 60-66.

Anderson, G. B. 1998. Embryonic stem cells in agricultural species. In: Transgenic Animals in Agriculture (Ed. J. D. Murray, G. B. Anderson, A. M. Oberbauer and M. M. McGloughilin). CAB Int., London. pp. 57-66.

Arima, T., F. Ebara, H. Furuta and N. Fujihara. 2000. Possible factor of chicken spermatozoa for transferring foreign gene into avian embryos. Proc. IIIrd Asian Ocea. Congr. Androl., Chiba. (in press).

Bakst, M. R. 1990. Preservation of avian cells. In: Poultry Breeding and Genetics (Ed. R. D. Crawhord). Elsevier, Amsterdam. pp. 91-108.

Bourne, J. 1999. The organic revolution. Audubon. 101:64-70.

Cadbury, D. 1997. The Feminization of Nature: Our Future at Risk. Hamish Hamilton Ltd., London.

Carson, R. 1982. Silent Spring. Penguin, Harmondsworth, UK.

Colborn, T., D. Dumanoski and J. P. Myers. 1997. Our Stolen Future. A Plume/Penguin Book, New York.

Crawford, R. D. 1990. Poultry genetic resources: evolution, diversity, and conservation. In: Poultry Breeding and Genetics (Ed. R. D. Crawhord). Elsevier, Amsterdam. pp. 43-60.

Delany, M. E. and J. M. Pisent. 1998. Conservation of poultry genetic research resources: Consideration of the past, present and future. Poult. Avian Biol. Rev. 9:25-42.

Dobson, A. P. Conservation and Biodiversity. 1998. Scientific American Library, New York.

Ebara, F. and N. Fujihara. 1999. Reproductive characteristics of transgenic (TG) chickens carrying an exogenous gene. Asian J. Androl. 1:139-144.

Ebara, F. and N. Fujihra. 1999. *In vivo* transfer into chicken embryos via primordial germ cells using green fluorescent protein as a marker. J. Reprod. Dev. 45:(in press).

Eguma, K., T. Soh, M. Hattori and N. Fujihara. 1999. *In vitro* transfer of foreign DNA into primordial germ cells (PGCs) of chicken embryos. Aisan-Aus. J. Anim. Sic. 12:520-524.

Fan, G. L., N. Fujihara, H. C. Zhou, Y. P. Xu, Y. H. Chao and W. K. Fu. 1999. Observation on the morphological structure of some immunological organs of crested ibis. Proc. Int. Workshop Crested Ibis, Hanzhong. pp. 133-136.

Fan, G. L., J. F. Qian, H. C. Zhou, Y. H. Chao, W. K. Fu,

B. H. Lu and Y. M. Xi. 1999. Observation on the morphological structures of some endocrine organs of crested ibis. Proc. Int. Workshop Crested Ibis, Hanzhong. pp. 126-129.

Fan, G. L., Y. M. Xi, H. C. Zhou, Y. H. Cao, W. K. Fu, B. Z. Lu, Y. Nakaya and N. Fujihara. 2000. Pathological Characteristics of a Dead Domestic Crested Ibis in China. Jpn. J. Zoo Med. Wild. 5:85-89.

Fang, S. G., W. H. Feng, A. J. Zhang, S. C. Li, G. H. Li and J. Q. Yu. 1998a. Genetic diversity of the population of giant pandas in Qinling Mountains detected by F2 Probe. Proc. Int. Symp. Pro. Giant Panda, Chengdu. pp. 134-140.

Fang, S. G., W. H. Feng, A. J. Zhang, H. W. Chen, J. Q. Yu, L. S. He and N. Fujihara. 1998b. The research on genetic diversity of the gaint pandas. Proc. Int. Symp. Pro. Giant Panda, Chengdu. pp. 141-153.

Fang, S. G., W. H. Feng, A. J. Zhang, S. C. Li, J. Q. Yu, X. M. Huang and G. G. He. 1997a. The comparative analysis on the genetic diversity of giant pandas between liangshan and Xiaoxiangling Mountain. Acta. Therio. Sinica. 17:248-252.

Fang, S. G., M. Huang, G. Q. Cheng and C. W. Din. 1997b. The preparation of fish oligonucleotide fingerprinting probe. Acta Gen. Sinica. 24:7-14.

Fang, S. G., W. H. Feng and A. J. Zhang. 1997c. The developing of giant panda DNA fingerprinting probe F2 and the comparative test analysis of 5 probes. Acta Therio. Sinica. 17:165-171.

Fang, S. G., W. H. Feng, A. J. Zhang, S. C. Li and J. Q. Yu. 1997d. DNA fingerprinting analysis on the paternity determined of giant panda. Acta Therio. Sinica. 17:92-99.

Fang, S. G., J. H. Lu, C. F. Xu, J. Tang and J. A. Zhang. 1997e. Detecting the DNA fingerprints of a throng in Chengdu with oligonucleotide probe LZF-1. Chin. J. Appl. Env. Biol. 3:41-43.

Fang, S. G., W. H. Feng, A. J. Zhang, S. C. Li and T. Q. Yu. 1997f. DNA fingerprints on the paternity determination of artificial breeding giant pandas. J. Sichuan Univ. 34:78-82.

Farrell, D. J. 1995. The table egg laying ducks: Nutritional requirements and current husbandry systems in Asia. Poul. Avian Biol. Rev. 6:55-69.

Feng, W. H. and J. C. Fu. 1994. Giant Panda Conservation. Min. Int. Symp. Prot. Giant Panda, Chengdu, Sichuan Pub. House Sci. Technol.

Feng, W. H. and A. J. Zhang. 1991. A Study on Breeding and Disease of the Giant Panda (*Ailuropoda melanoleuca*). Sichuan Pub. House Sci. Technol.

Feng, W. H. and G. X. He. 1991. Studies on the Giant Panda. J. Sichuan Univ. Natr. Sci. 28:1-183.

Feng, W. H., J. Zhao and N. Fujihara. 1996. Natual conservation and research trend of the Giant Panda (*Ailuropoda melanoleuca*). Jpn. J. Zoo Wild. Med. 1:135-142.

Feng, W. H., J. Zhao and N. Fujihara. 1997. Physiological characteristics of electro-ejaculated giant pana (*Ailuropoda melanoleuca*). semen. Jpn. J. Zoo Wildl. Med. 2:107-112.

Fujihara, N. 1994. Present status of researches on *in vitro* preservation of avian spermatozoa. In: Research report of preservation and reutilization of genetic resources from endangered wild animals. Waseda Univ., Human Res. Cent., Tokyo. pp. 54-62.

Fujihara, N. 1996. *In vitro* preservation and reutilization of spermatozoa from domestic animals. Por Terk. 69:33-47.

Fujihara, N. 1996. Preservation and reutilization of genetic resources f rom endangered animals in the world. New Food Ind. 39:27-32.

Fujihara, N. 1997. Comparative physiology of semen and spermatozoa in avian species. Cur. Top. Pharmacol. 3:53-63.

Fujihara, N. 1998a. Functional diversity of avian spermatozoa. Poult. Res. 73:61-65.

Fujihara, N. 1998b. Application of basical animal science studies to the restoration of endangered and/or extinct wild animals in the world. Trend Sci. 3:58-61.

Fujihara, N., S. Inada, M. Hasebe, H. Yamaguchi and M. Hattori. 1998. *In vivo* gene transfer to chick embryos via spermatozoa and primordial germ cells. In: Reproductive Biology Update (Ed. H. Miyamoto and N. Manabe). Nakanishi Print. Ltd, Kyoto. pp. 389-401.

Fujihara, N. 1999a. Poutry genetic resources and conservation biology - review. Jpn. Poult. Sci. 36:127-147.

Fujihara, N. 1999b. Possible production of transgenic chicken by transferring foreign genes and germ cells - review. K. J. Anim. Sci. 26:119-127.

Fujihara, N. 1999c. Preservation and reutilization of genetic resources from useful animals in the world. New Food Ind. 41:49-55.

Fujihara, N. and Y. M. Xi. 1999d. Genetic resource conseration in the waterfowl. Proc. Int. Conf. Waterfowl, Taiwan. pp. 78-84.

Furuta, H., H. Yamaguchi and N. Fujihara. 1999. Development of the gonads derived from hetero-sexually transferred primordidal germ cells (PGCs) between embryos in the chicken. Asian-Aus. J. Anim. Sci. 12:1188-1191.

Furuta, H. and N. Fujihra. 2000. Proliferation of exogenously introduced primordial germ cells (PGCs) into the buslfun-treated chicken embryos. Asian J. Androl. 1:(in press).

Furuta, H., K. B. Kim and N. Fujihara. 2000. *In vivo* gene transfer into chicken blastoderm by lipofection and electroporation. J. Appl. Anim. Res. 15:(in press).

Goto, K. 1998a. Possible establishment of mammoth park in Siberia. Newton. 18:110-117.

Goto, K. 1998b. The Project for Reviving Mammoth. Kodansha Pub. Co., Tokyo.

Goto, K. 1998c. The Revival of Mammoth in the World. Kodansha Pub. Co., Tokyo.

Goto, K. 1998d. The Day for Reviving of Mammoth in the World. Futaba Ltd., Tokyo.

Hackett, P. B., Z. Izsvak, Z. Ivics and L. Caldovic. 1999. Development of genetic tools for transgenic animals. In: Transgenic Animals in Agriculture (Ed. J. D. Murray, G. B. Anderson, A. M. Oberbauer and M. M. McGloughilin), CAB International, London. pp. 1-18.

Hagino, N. 1996a. Kampo medicine and neuroendocrine - From here to molecular biology, J. Trad. Med. 13:105-117.

Hagino. N. 1996b. Kampo medicine is the potential therapeutics for treatment of dementia of alzheimer type. Prog. Med. 16:73-80.

Hasebe, M., T. Soh, M. Hattori and N. Fujihara. 1998. An

attempt to produce transgenic chicken mediating sperm cells as vectors. J. Appl. Anim. Res. 14:143-150.

Higuchi, H. 1996. Conservation Biology. Tokyo Univ. Press, Tokyo.

Iguchi, T., S. Tanabe, T. Horiguchi, L. J. Guillette, T. B. Hayes, D. M. Sheehan, K. Arizono, Y. Kuroda, A. Hara and H. Kaneko. 1998. The present situation of environmental hormones. Sci. J. KAGAKU. 68:529-605.

Inada, S., M. Hattori, N. Fujihara and K. Morohashi. 1997. *In vivo* gene transfer i into the blastoderm of early developmental stage of chicken. Reprod. Nutr. Dev. 37:13-20.

Inada, S., M. Hattori and N. Fujihara. 1999. *In vivo* gene tansfer to chicken via blastodermal cells of early developmental embryos. In: Animal Cell Technology: Challenge for the 21st Century (Ed. K. Ikura, M. Nagano, S. Masuda and R. Sasaki). Kluwer Academic Pub., Dordrecht. pp. 208-213.

Ishii, S. 1994. Preservation and reutilization of genetic resoruces from endangered animals. Res. Rep. Waseda Univ. Human Res. Center, Tokyo. pp. 1-82.

Ishijima, I., T. Oishi, T. Nagashima and M. Yokohama. 1997. Biological Resources and its Utilization. Sankyo Pub., Tokyo.

Ives, C. L. and B. M. Bedford. 1998. Agricultural Biotechnology in International Development. CAB Int., London.

Kagami, H. and H. Hanada. 1997. Current knowledge of sexual differentiation in domestic fowl. World's Poult. Sci. J. 53:111-123.

Kagami, H., T. Tagami, Y. Matsubara, T. Harumi, H. Hanada, K. Maruyama, M. Sakurai, T. Kuwana and M. Naito. 1997. The developmental origin of primordial germ cells and the transmission of the donor-derived gametes in mixed-sex germline chimeras to the offspring in the chicken. Mol. Reprod. Dev. 48:501-510.

Kawabata, H. 1998. Challenging zoo in America. Scias. No. 46:36-41.

Klingholz, R. 1998. Protecting the forest in Nepal. Int. Mag. GEO. 5:70-79

Koyama, T., M. Ohara, M. Ichimura and M. Saito. 1988. Effect of Japanese Kampo Medicine on hypothalamic-pituitary-ovarian function in women with ovarian insuffficiency. Am. J. Chinese Med. 16:47-55.

Kunimatsu, T. 1999. The Last One of the Crested Ibis in Japan. Kinnohoshi Pub. Co., Tokyo.

Kuwana, T. 1993. Migration of avian primordial germ cells towards the gonadal anlage. Dev. Growth Diff. 35:237-243.

Liu, S. X., Z. Z. Qin and Y. M. Xi. 1997. New finding of paracites special to crested ibis. J. Anim. Taxon. 22:6-9.

Liu, S. X., Y. M. Xi and Y. J. Wang. 1995. Paracitological experiments in the guts of newborn crested ibis. Sichuan Anim. 14:133-136.

Long, M. 1998. The vanishing prairie dog, Nat. Geogr. 193:116-130.

Lukaszewicz, E., H. Furuta, Y. M. Xi and N. Fujihara. 2000. Analysis of semen quality of 1 and 2 years old ganders during the entire reproductive season. Asian J. Androl. 1:(in press).

Naito, M., A. Tajima, Y. Yasuda and T. Kuwana. 1994. Production of germline chimeric chickens, with high transmission rate of donor-derived gametes, produced by transfer of primordial germ cells. Mol. Reprod. Dev. 39:153-161.

Nakaya, Y. and N. Fujihara. 1999. An introduction of Chengdu Research Base for Giant Pandas. Anim. Husb. 54:256-262.

Obara, H. 1998. The vanishing animals in Japan. Newton. 18:26-41.

Obara, H. and T. Hotta. 1998. Paradise of life. Newton. 18:58-81.

Obara, H. 1999a. Ecosystem in danger of extinction. Newton. 19:32-43.

Ohboshi, S. 1999. Cryopreservation of mammalian embryos by vitrification - review. Anim. Sci. Technol. 69:1069-1077.

Okada, M. and Y. Nagahama. 1996. Germ Cells. Kyoritsu Pub. Ltd., Tokyo.

Otoi, T., K. Yamamoto, N. Koyama, S. Tachikawa and T. Suzuki. 1996. A frozen-thawed *in vitro*-matured bovine oocytes derived calf with normal growth and fertility. J. Vet. Med Sci. 58:811-813.

Petitte, J. N., S. D'Costa and L. Karagenc. 1998. Understanding the origin of avian primordial germ cells: Implications for germ cell culture and transgenic in poultry. In: Transgenic Animals in Agriculture (Ed. J. D. Murray, G. B. Anderson, A. M. Oberbauer and M. M. McGloughilin). CAB International, London. pp. 97-116.

Pinkert, C. A. and J. D. Murray. 1998. Transgenic farm animals. In: Transgenic Animals in Agriculture (Ed. J. D. Murray, G. B. Anderson, A. M. Oberbauer and M. M. McGloughilin). CAB Int., London. pp. 1-18.

Primack, R. B. 1995. A Primer of Conservation Biology. Sinauer Associates Inc., Massachusetts.

Primack, R. B. 1998. Essentials of Conservation Biology. Sinaur Associates Inc., Massachusetts.

Reiss, M. J. and R. Straughan. 1996. Improving Nature?- The Science and Ethics of Genetic Engineering. Cambridge University Press, London.

Romanov, M. N., S. Wezyk, K. Cywa-Benko and N. I. Sakhatsky. 1996. Poultry genetic resources in the countries of Eastern Europe - History and current state. Polut. Avian Biol. Rev. 7:1-29.

Sato, K. 1998. Role of Kampo Medicine in obstetrics and gynecology. Recent. Prog. Kampo Medicine in obstet. gynec. 15:1-8.

Schaller, G. B., J. C. Hu, W. S. Pan and J. Zhu. 1985. The Giant Pandas of Wolong, University Chicago Press, Chicago.

Shiva, V. 1997. Monoculture of the Mind. Third World Network, Malaysia.

Squires, E. J. 1998. Status of sperm-mediated delivery methods for gene transfer. In: Transgenic Animals in Agriculture (Ed. J. D. Murray, G. B. Anderson, A. M. Oberbauer and M. M. McGloughilin). CAB International, London. pp. 87-95.

Tagami, T., Y. Matsubara, H. Hanada and M. Naito. 1997. Differentiation of female chicken primordial germ cells into spermatozoa in male gonads. Develop. Growth Differ. 39:267-271.

Tanabe, Y., H. Nigi, Y. Fujimaki and M. Yoneda. 1996. An Introduction to Wild Animal Sciences, Asakura Shoten, Tokyo.

Washitani, I. 1999. Ecology for Biological Conservation. Kyoritsu Publishing, Tokyo.

Watson, P. F. 1978. Artificial Breeding of Non-Domestic Animals. Academic Press, London.

Williams, T. 1999. Managment by majority. Auddubon. 101:40-49.

Wilmut, I., E. Schnieke, J. McWhir, A. J. Kind, A. Colman and K. H. S. Campbell. 1998. Nuclear transfer in the production of transgenic farm animals. In: Transgenic Animals in Agriculture (Ed. J. D. Murray, G. B. Anderson, A. M. Oberbauer and M. M. McGloughilin). CAB International, London. pp. 67-78.

Wong, E. A., A. L. Wentworth, B. C. Wentworth, J. A. Proudman and M. E. El. Halawanil. 1998. Generation of transgenic poultry by transfection of primordial germ cells. In: Transgenic Animals in Agriculture (Ed. J. D. Murray, G. B. Anderson, A. M. Oberbauer and M. M. McGloughilin). CAB International, London. pp. 117-129.

Xi, Y. M., B. Z. Lu and W. K. Fu. 1995. Rescuing and conservation of newborn crested ibis. Wild Anim. 16:37-41.

Xi, Y. M., B. Z. Lu, Z. Z. Geng, T. Q. Zhai and Y. M. Zhang. 1997. Conservation of crested ibis. Wild Anim. 18:28-30.

Xi, Y. M., B. Z. Lu, Z. Z. Geng, T. Q. Zhai, Y. M. Zhang and L. Huang. 1999a. Feeding and breeding studies of crested ibis. Proc. Int. Work. Crested Ibis Conser., Hanzhong. pp. 102-108.

Xi, Y. M., W. K. Fu and B. Z. Lu. 1999b. Clinical observation and curing of special diseases of crested ibis. Proc. Int. Works. Crested Ibis Conser., Hanzhong. pp. 78-84.

Xi, Y. M. and N. Fujihara. 2000a. The future of crested ibis in China and Japan. Jpn. J. Zoo Wildlfe Med. 5:107-112.

Xi, Y. M. and N. Fujihara. 2000b. Present status of conservation of creseted ibis in China. Jpn. J. Human Anim. Rel. 6:(in press).

Yamaguchi, H., Y. M. Xi and N. Fujihara. 2000. Inter-sexual transfer of primordial germ cells (PGCs) between chicken embryos. Cytotechnol. 33:(in press).

Yang, G. Q. and N. Fujihara. 1999a. Long-term proliferation of chicken primordial germ cells cultured *in vitro*. J. Reprod. Dev. 45:161-166.

Yang, G. Q. and N. Fujihara. 1999b. Survival and proliferation of refined chicken circulating primordial germ cells culture *in vitro*. J. Reprod. Dev. 45:177-181.

Zhang, A. J. and G. X. He. 1998. Natural and Captive Conservation of Giant Pandas, Proc. Int. Symp. Environ. Prot. City Dev. 21st Cent., Chnegdu, Sichuan Pub. House Sci. Technol. Ltd.

Zhao, J., M. A. Hattori and N. Fujihara. 1997. Effect of Japanese Kampo Medicines on physiological function of frozen-thawed bovine spermatozoa in *in vitro* fertilization. J. Mamm. Ova Res. 14:169-174.

Zhao, J., M. A. Hattori and N. Fujihara. 1998a. Effects of Japanese Kampo Medicines (JKMs) on *in vitro* maturation and subsequent embryonic development of bovine oocytes. J. Appl. Anim. Res. 13:93-104.

Zhao, J., M. A. Hattori and N. Fujihara. 1998b. *In vitro* maturation of oocytes and developmental ability of embryos in *in vitro* fertilization of the cattle. In: Reproductive Biology Update (Ed. H. Miyamoto and N. Manabe). Nakanishi Print., Kyoto. pp. 189-195.

Zirkin, B. R. 1998. Germ Cell Development, Division, Disruption and Death. Springer -Verlag, New York.