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Endocrinological studies for artificial breeding of the Japanese ibis, *Nipponia nippon*, an endangered avian species in Asia

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Abstract. Once the Japanese ibis, or the Japanese crested ibis, was widely distributed in Asia including Japan, Korea, China and Siberia, and was not a rare species. However, this species started to disappear over its entire range beginning in the late 19th or early 20th century. Currently, only a single population of 15-20 individuals survives in wild in Yang Xian, Shaanxi, China. Several individuals, mostly immature birds, are kept in captivity in Beijing zoo. One of them is an adult male captured in 1981 in Japan and sent to Beijing zoo for breeding two years ago. In Japan, only, a single old female survives in captivity. Scientists of the Japanese Ibis Preservation Center in Sado Island and Ueno zoo, Tokyo, had attempted several times to breed Japanese ibises in captivity, but they have failed in all of their attempts. In Beijing zoo, a similar attempt is now being carried out.

As the basis of an artificial breeding programme of this and other species of birds, the authors have attempted to establish a noninvasive method for estimation of gonadal activities of birds and also a method to induce a complete series of the ovarian activity, *i.e.,* ovarian growth, ovulation and oviposition, by means of hormone administration to some species of birds. In this communication, the author briefly reports recent results of these attempts in addition to results of measurements of gonadotropin levels in plasma of captive Japanese ibises and white ibises, a closely related species, *Threskiornis aethiopicus.*

Keywords. *Nipponia nippon;* artificial breeding; endangered bird; gonadotropin; fecal sex Steroids.

1. Introduction

The history of extinction of wild populations of Japanese ibises in Asia and the current status of the last wild population and captive individuals of this species will be described. Furthermore, our endocrinological attempts to establish an artificial breeding programme for this species will be described. It is hoped that this programme will not be too late to save this species.

2. The Japanese ibis

The Japanese ibis or Japanese crested ibis (*Nipponia nippon*), a beautiful bird

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belonging to the family Threskiornithidae of the order Ciconiiformes, was first described by Temminck (1835) as *Ibis nippon.* According to an extensive review edited by Yamashina and Nakanishi (1983), Japanese ibises were rather common in Japan and widely distributed over most parts of Honshu (the main Island), a part of Hokkaido and four small islands, *i.e.,* Sado, Oki, Ohshima and Hachijohjima at least in 18th Century. In 19th Century, they expanded their distribution to Kyushu and Shikoku, the other two big islands comprising Japan. However, Japanese ibises started to decrease in their numbers beginning in the late 19th Century, and were thought to be in complete extinct in early 20th Century in Japan. However, they were rediscovered in Noto Peninsula in 1929 and in Sado Island in 1930. Changes in the size of the wild populations in these two places between 1952 and 1980 are shown in figure 1. In January 1980, the last few wild individuals (four females and a male) of this species in Japan were captured in Sado Island, and kept in cages in the Sado Japanese Ibis Preservation Center, Niigata Prefecture, with a female that was captured in 1968 and had been kept there already.

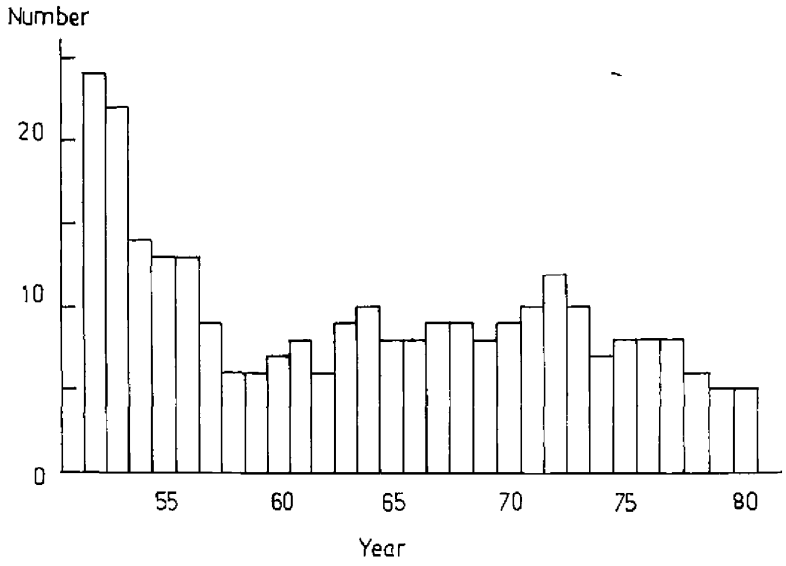

Figure 1. Change in the size of the wild population of the Japanese ibis in Sado Island between 1952 and 1981.

The review by Yamashina and Nakanishi (1983) also described that Japanese ibises were often observed in many places in Korea and in Primirskii, Siberia in the 19th and early 20th Century, but these populations also disappeared there after. The last observation of this species in Korea was in 1978 and that in Primorskii in 1981 according to this review.

In China, the situation was similar, and extinct of this species in China was going to be officially announced in 1981. Unexpectedly, in this year, two nesting pairs were discovered in Yang Xian, Shaanxi. The population size including young in this area in 1990 was estimated to be between 15-20 (Report of the Japanese Ibis Breeding Project, Niigata-Ken, 1990). This is the only known wild population of the Japanese ibis in the world.

Beside this single wild population, several living individuals (the exact number unknown) of the Japanese ibis, mostly young and including an adult male sent from the Sado Japanese Ibis Preservation Center, are kept in captivity in the Beijing zoo. A single old female (estimated to be 24 years old now) also survives in captivity in the Sado Japanese Ibis Preservation Center. The other individuals captured in 1980 and maintained in the Center have died.

3. Trials for breeding Japanese ibis in captivity in Japan

There were six Japanese ibises kept in captivity in the Sado Japanese Ibis Preservation Centre in 1981. Five of them were females (one named "Kin" was 14 years old, and the others were younger but exact ages were unknown) and the remaining was a male named "Midori". Two females died within 1981. In March of 1982, one, named "Shiro", of the two younger females was placed with the male, Midori, in the same cage for breeding. The other younger female named "Ao" was annoyed with arthritis on one of her legs and was not healthy. She had never been used for breeding before she died in 1986. Although Shiro and Midori displayed typical breeding behaviour during the breeding season in 1982, no copulation was observed this year. In the spring of 1983, copulation was frequently observed, and egg laying was expected. However, Shiro suddenly died without laying. It was revealed by autopsy that an egg was stuck in the oviduct and this was presumed to be the cause of her death.

After 1983, the old female, Kin, was paired with Midori almost every year until 1989. Kin was judged to be sexually active from her behaviour and nuptial colouration at least during the earlier two thirds of this period. However, breeding was unsuccessful.

It is important to consider the reason why Japanese scientists failed in their attempts to breed Japanese ibis in captivity. Two related species, sacred ibis (*Threskiornis melanocephalus*) and scarlet ibis (*Eudocimus ruber*), are breeding in captivity in the Tama zoo, Tokyo. This fact suggests a possibility that the Japanese ibis can breed in captivity. The finding of an egg in the dead female in 1983 also supports this possibility. However, we have not enough Japanese ibises to prove it in Japan. Chinese scientists have been successful in captive breeding of the Japanese ibis recently in Beijing Zoo and have two and three young ones hatched in 1992 and 1993 respectively.

4. Roles of endocrinological methods in captive breeding

It is important to know sexual maturity and onset of breeding activity of each individual bird in order to breed birds successfully in captivity. Nuptial colouration and sexual behaviour are useful indices of these states. However, a more important and reliable index is hormone levels in blood plasma. Increases of levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in plasma are a good indices of sexual maturity and onset of breeding in both sexes. In addition, ovulation in females can be detected by a sudden rise in the plasma LH level referred to as a "LH surge".

4.1 *Annual cycle of gonadotropins in plasma in the sacred ibis*

Since frequent sampling of blood was not allowed in the captive individuals of the Japanese ibises, we employed male and female sacred ibises reared in Tama zoo, Tokyo as research animals, and surveyed monthly changes in LH (figure 2a and b) and FSH levels (figure 2c and d) in their plasma (Ishii 1983, 1984). The species breed in captivity in the Tama zoo from spring to summer. For the measurements, we used radioimmunoassays (RIAs) developed by ourselves using specific antisera for chicken FSH and LH. Level of LH showed the highest peak in February or March with lower peaks in December and June. FSH also showed similar changes except that the highest peak was observed in June. Thus, it is obvious that FSH and LH in plasma of the sacred ibis can be measured by the RIAs we employed, and also that we can use FSH and LH levels in plasma as useful indications of breeding activity in this species of ibis.

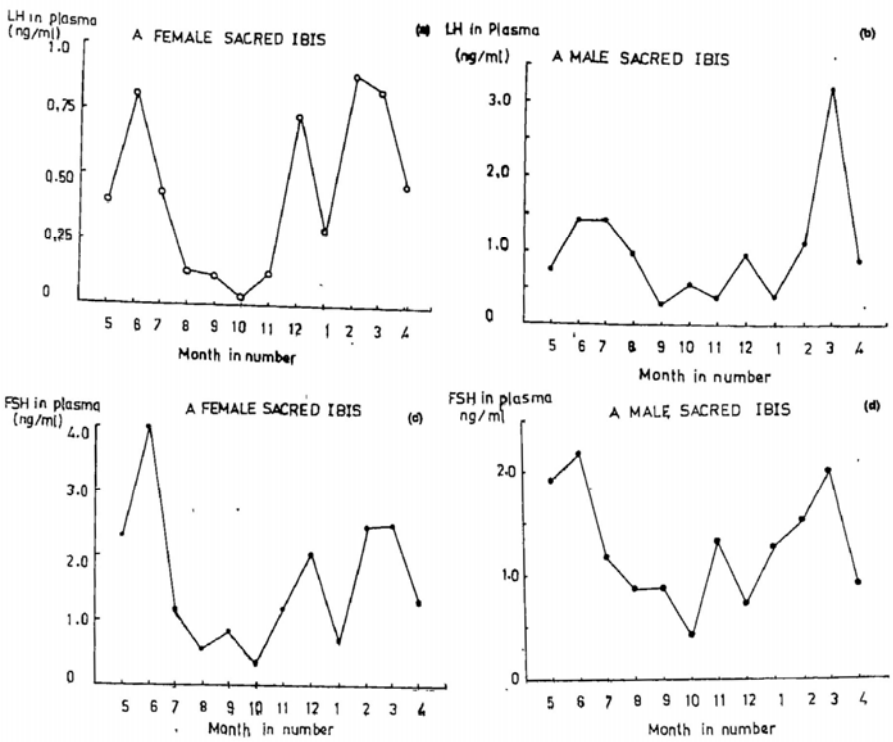

Figure 2. Monthly changes in gonadotropin levels in the plasma in the sacred ibis. **(a)** LH in a female; **(b)** LH in a male; **(c)** FSH in a female; **(d)** FSH in a male.

4.2 *FSH and LH levels in plasma of Japanese ibises*

Fortunately, we had been able to obtain small amounts of blood plasma from three females (Ao, Kin and Shiro) and a male (Midori) of the Japanese ibis in October 1982 (Ishii 1984). Their LH levels differed largely among individuals, while FSH levels were closer (figure 3). The LH level was highest in a young adult female

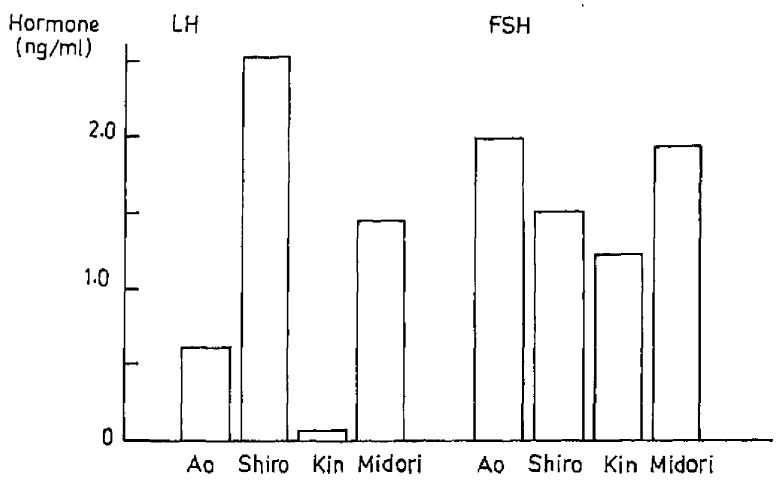

Figure 3. LH and FSH levels in plasma in three females (named Ao, Shiro and Kin) and a male (named Midori) of the Japanese ibis in October 1982.

(Shiro) which died later bearing an egg in the oviduct, and lowest in the oldest female (Kin). The LH level in the male (Midori) was intermediate, These results indicate that LH and FSH levels in blood plasma of the Japanese ibis can be measured by our RIAs, and plasma LH level was extremely low in the oldest female.

4.3 *Noninvasive method to estimate gonadal endocrine activity in birds*

Since frequent collection of blood samples should be avoided if at all possible in rare birds such as Japanese ibis, we had to find a noninvasive method to estimate gonadal endocrine activity as an alternative to the plasma hormone analysis. In 1985, Professor Ishii in collaboration with Dr Masaru Wada undertook a series of investigations to determine whether fecal hormone analysis could be used as a noninvasive alternative of plasma hormone analysis (Ishii 1985; Ishii *et al* 1986). We employed the Japanese quail as material, and compared concentrations of estradiol-17β (in females) and testosterone (in males) in the plasma and in the feces of the quail under different conditions (tables 1 and 2). In this experiment, we used a saline extract of feces as the assay sample. Whenever the plasma steroid concentration increased, the fecal steroid concentration also increased, Kubodera *et al* (1988) tested effects of implantation of progesterone and estradiol-17β on

Table 1. Concentrations of estradiol-17â in blood plasma and feces of female Japanese quail in different conditions,

Conditions		Estradiol concentration	
Animal	Photoperiod	Plasma (pg/ml)	Feces (pg/dropping)
Immature	8L, 16D	0·05 ± 0·02	4·146 ± 1·26
Mature	8L, 16D	3·09 ± 1·31	16·30 ± 5·32
Mature	16L, 8D	8·89 ± 3·35	341·9 ± 98·0

Table 2. Concentrations of testosterone in blood plasma and feces of male Japanese quail in different conditions.

Conditions		Testosterone concentration	
Animal	Photoperiod	Plasma (pg/ml)	Feces (pg/dropping)
Mature	8L, 16D	9·3 ± 4·9	21·9 ± 2·7
Mature	16L, 8D	328·3 ± 60·5	85·9 ± 19·8
Castrated		3·2	3·8 ± 0·8

concentrations of these steroids in plasma and feces of ovariectomized Japanese quail. Scilastic tubes containing sex steroids were implanted. To change the amount of steroid administered, each bird was implanted once a week for three weeks with a tube containing steroid. Sampling of blood and feces were performed daily for three successive days prior to each implantation. Concentrations of steroids in plasma and feces were expressed as the mean of three samples representing the three days (figures 4 and 5). In both plasma and feces, the estradiol concentration

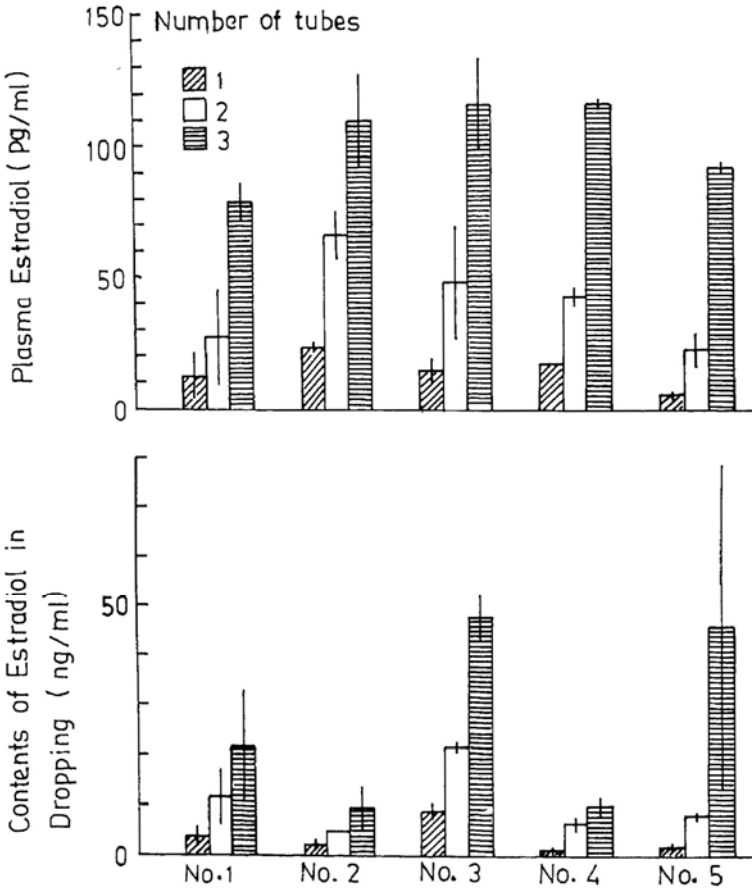

Figure 4. Effects of implantation of increasing number of scilastic tubes containing estradiol on plasma (upper figure) and fecal (lower figure) estradiol levels in a female Japanese quail. Individual number is indicated under the horizontal axis.

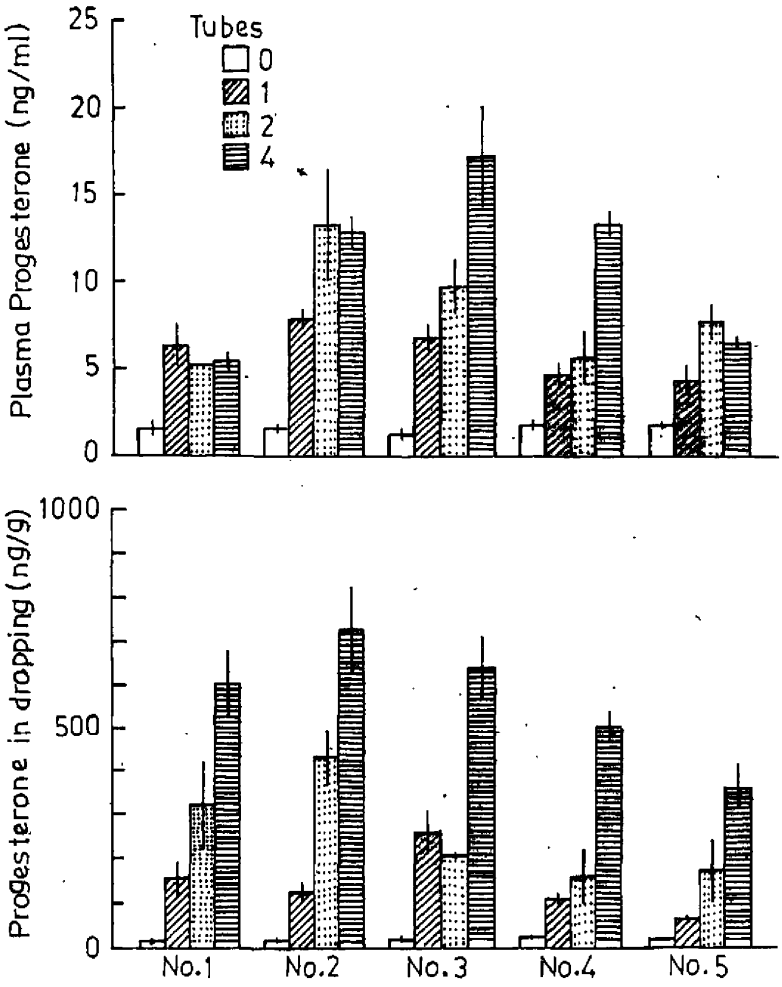

Figure 5. Effects of implantation of increasing number of scilastic tubes containing progesterone on plasma (upper figure) and fecal (lower figure) progesterone levels in a female Japanese quail. Individual number is indicated under the horizontal axis.

was increased as the number of tubes in the body was increased. The progesterone concentration also showed similar changes in both plasma and feces to the estradiol concentrations. These results indicate that plasma sex steroid concentrations are reflected in fecal sex steroid concentrations, although individual variation was larger in the feces than in the plasma.

4.4 *Can ovulation be detected by fecal steroid analysis?*

We were interested in knowing whether ovulation can be detected by fecal steroid analysis. Recently, Yamaguchi and Ishii (1991) demonstrated in normal laying females of the Japanese quail that the progesterone concentration in the feces shows a conspicuous rise around the time of ovulation (figure 6). Estradiol and testosterone did not show such a change. Thus, ovulation can be detected by monitoring fecal progesterone concentration.

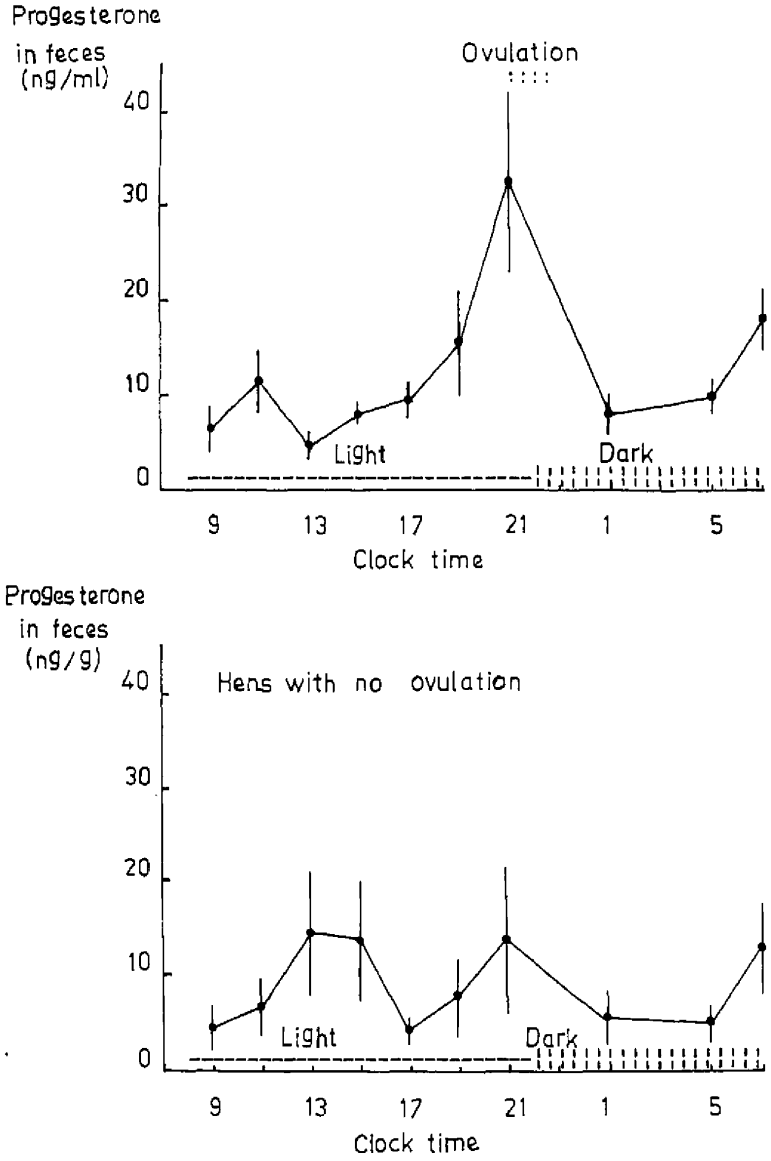

Figure 6. Changes in mean fecal progesterone levels in laying (upper figure) and non-laying hens (lower figure) of the Japanese quail within a day. Estimated time of ovulation is indicated in the upper figure. Vertical bars indicate the standard error of the mean.

4.5 *Hormonal induction of ovarian growth and ovulation in Japanese quail*

One of the two female Japanese ibises kept in the Sado Japanese Ibis Preservation Center did not lay any eggs, although she developed nuptial colour every year. We supposed two possibilities to explain this. One is that her ovary did not develop enough to contain mature follicles, and the other is that there was no LH surge to induce ovulation, although she developed mature follicles. Considering these two

possibilities, we attempted to induce ovarian growth followed by ovulation by hormone administration in female Japanese quail kept under short-day conditions. A number of studies have already been published on the hormonal induction of ovarian growth and ovulation in birds. Some of the investigators reported that they could induce ovarian growth by gonadotropin administration to hens with a completely regressed ovary, but no ovulation was induced except a single individual case in the domestic hen. Other investigators using newly hypophysectomized or pharmacologically treated hens with fully grown ovary could only induce ovulation in such birds. None of these investigators have been able to induce both ovarian growth and ovulation in hens with a completely regressed ovary except the single individual case mentioned above.

In preliminary experiments, we found that it was difficult to maintain plasma gonadotropin concentrations at a level high enough to stimulate ovary for a long time by injecting gonadotropins. Then, we employed "ALZET Osmotic Pump" that releases its content at a constant rate for one to two weeks. When an osmotic pump, ALZET Model 2002 whose size is 3·0 × 0·7 cm and output speed is 0·5 µl/h, was filled with a chicken pituitary glycoprotein solution (0·5 mg/ml or 2·5 mg/ml) and implanted into peritoneal cavity of an adult female Japanese quail, we could maintain high plasma gonadotropin levels for almost two weeks (figure 7) (Wakabayashi *et al* 1987). We could induce development of the ovary of Japanese quail kept in short-days to the size normally observed in laying hens of this species by implanting an osmotic pump filled with a chicken pituitary glycoprotein fraction (table 3). However, they did not lay. Then, we combined these two routes of administration of gonadotropin. Using female Japanese quail three weeks old kept in short-days, the osmotic pump filled with the chicken pituitary glycoprotein fraction was implanted for two weeks, and then the chicken pituitary glycoprotein fraction was injected daily for five successive days. Three out of 7 treated hens laid 8 eggs in total. Hens were kept with males for a certain period once a day during the injection period. By incubating the eggs artificially, 2 out of the 8 hatched. The chicks were males, and grew normally. At least one of them was fertile.

Thus, we could establish a method to induce full development of the ovary followed by ovulation in female Japanese quail with completely regressed ovary by hormonal treatments.

4.6 *Effects of chicken gonadotropin and luteinizing hormone releasing hormone on the ovary and oviduct of the Chinese grosbeak*

The pituitary glycoprotein used in our study was of chicken origin, and the bird treated was the Japanese quail, *Coturnix coturnix japonicus.* Both donor and recipient belong to the same major taxonomic group within the birds, *i.e.*, the Galliformes. Accordingly, we supposed that the possibility existed that pituitary glycoprotein or gonadotropin of chicken origin would not be active on the ovary of birds of different major groups, *e.g.*, the Ciconiiformes. To test this possibility, Wakabayashi *et al* (1987) treated Chinese grosbeaks, *Eophona migratoria,* members of the order Passeriformes, with the chicken pituitary glycoprotein and luteinizing hormone-releasing hormone of the mammalian type by means of the osmotic pump. As shown in

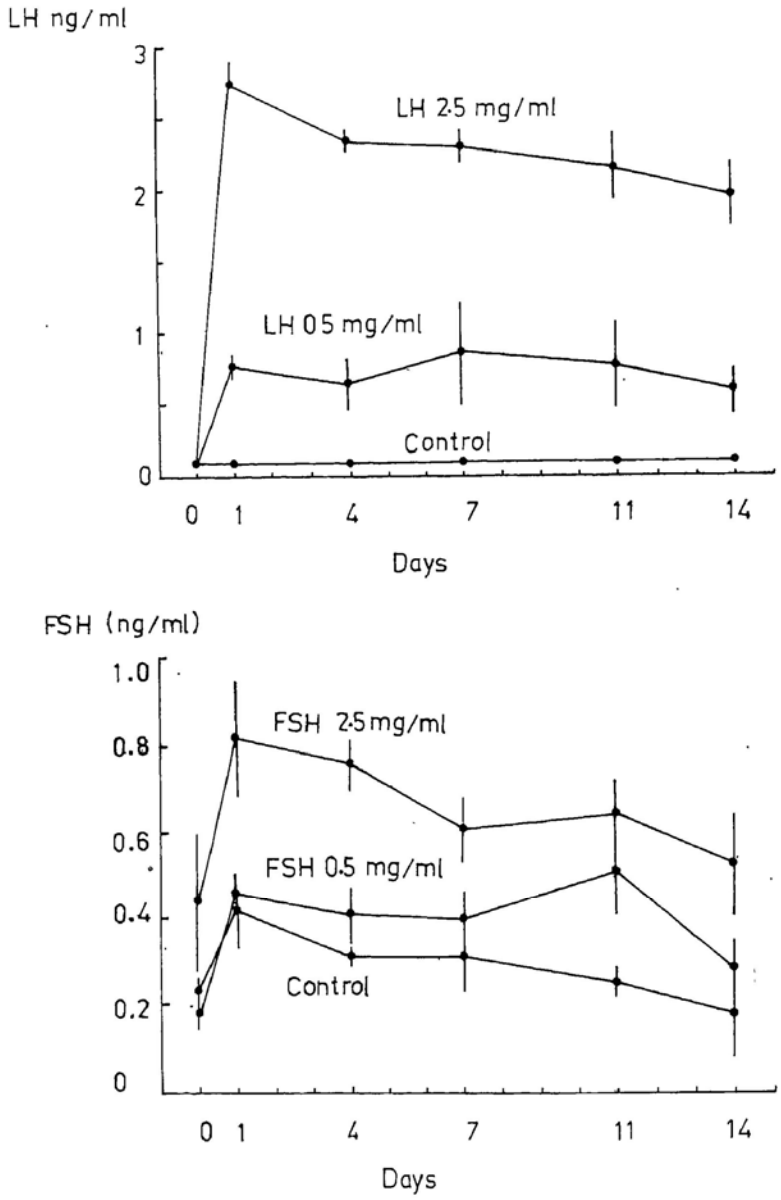

Figure 7. Effects of implantation of a osmotic pump containing two different concentrations of chicken pituitary glycoprotein in saline or saline alone (control) on mean plasma LH (upper figure) and FSH levels in female Japanese quail. Vertical bar indicate the standard error of the mean.

table 4, a high dose of the chicken pituitary glycoprotein was able to stimulate the ovary and oviduct of this species. Accumulation of yolk was observed in 7 of 9 individuals treated with the high dose of the glycoprotein. We may conclude that chicken gonadotropin is active on the passerine ovary.

Table 3. Effects of implantation of an osmotic pump containing a chicken pituitary glycoprotein solution (25 mg/ml) on the weight of the ovary and oviduct in female Japanese quail kept in short-days.

Group	Photoperiods	No. of birds	Ovarian weight (g)	Oviductal weight (g)
Control	Short-days	6	0·034 ± 0·007	0·055 ± 0·010
Control	Long-days	7	5·04 ± 0·68	4·91 ± 0·84
Treated	Short-days	3	6·34 ± 1·31	12·5 ± 3·3

Table 4. Effects of implantation of an osmotic pump containing a chicken pituitary glycoprotein solution (5 mg/ml or 25 mg/ml) on the ovarian weight, oviductal weight and diameter of the largest ovarian follicle in the Chinese grosbeak, *Eophona migratoria.*

Treatment	No. of birds	Ovarian weight (mg)	Oviductal weight (mg)	Diameter of the largest follicle (mm)
Saline	8	125·6 ± 51·5	216·1 ± 137·4	1·74 ± 0·55
Glp 5 mg/ml	10	120·6 ± 86·5	163·6 ± 52·3	1·80 ± 0·28
Glp 25 mg/ml	9	1259·9 ± 320·4	1419·7 ± 232·5	6·03 ± 0·82

4.7 *Effect of chicken gonadotropin on the ovary of some species of ibises*

In collaboration with Professor S Wakabayashi and Dr H Sakai of Nihon University, Professor M Wada of Tokyo Medical and Dental University, Dr M Kikuchi of Waseda University and Mr K Tanabe and Mr K Saito of Ueno zoo, we performed some preliminary experiments to stimulate the ovary of the sacred ibis, *Threskiornis melanocephalus,* and the scarlet ibis, *Eudocimus ruber.* However, due to the small number of birds available and a small amount of the chicken pituitary glycoprotein fraction relative to the size of the birds, we could not obtain reliable results. Further studies with a larger number of birds and a large quantity of gonadotropin are needed to know the effectiveness of chicken gonadotropin on the ovary of ibises.

5. Strategy for artificial breeding of the Japanese ibis

In the Beijing zoo, natural breeding in captivity is now being attempted by pairing a selected male and female. For this, analysis of fecal steroid is considered to be a useful procedure to determine the reproductive status of the individual ibises.

If estradiol and progesterone concentrations in feces are low in the female, she is considered to have undeveloped ovary. If she has high fecal levels of these ovarian sex steroids but still does not lay, lack of LH surge is suggested. In the former case, we may stimulate the ovary by gonadotropin administration using the osmotic pump followed by the injection of LH which stimulates LH surge. In the latter case, we may just inject LH to simulate LH surge for inducing ovulation. If androgen concentration in the male is abnormally low suggesting testicular regression, we may stimulate the testis by implanting an osmotic pump containing gonadotropin. The methods mentioned above have been completed and ready for

use, except that the incidence of induced ovulation is as low as 40% and validity of the methods in ibises has not been completely confirmed.

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