Chick Sexing with Excretory Steroids

Possible, Probable, Not Yet Practical

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Introduction

Watchbird readers have been informed about the historic developments of fecal steroid analysis and its utility as a non-invasive sexing method for adult birds (Bercovitz, 1981). Its potential for sexing newly hatched chicks was also discussed (Bercovitz et al., 1984). The AFA has had a direct involvement in this research, through its encouragement and financial support. Reported here are the summary results from our second AFA research grant (funded 10-23-84). It is an end to a beginning, success achieved with three captive-bred species and a solid foundation for applications to many other rare and endangered species. The only unfortunate aspect of this near ideal chick sexing method is indicated in the subtitle; it is *not yet* practical or available to AFA readers.

Egg waste material collection from day-old hatchlings is an excellent non-invasive sample. It includes everything but the shell and the chick. It is easily minced, liquified with buffer, and purified similar to droppings from adult birds. Only a brief general commentary is given in this article about hormonal sexing methods; Bercovitz (1987) reviewed and described all non-surgical sexing methods. Specific details about the analysis of excretory sex steroid hormones from adult bird feces has also been described (Czekala and Lasley, 1978; Bercovitz et al., 1978).

Experimental Research with "Model" Species

In my last review (Bercovitz et al., 1984), mention was made of successful *preliminary* sexing results from the analysis and interpretation of egg waste estradiol (E_2) measurement of hatchling California Condors *(Gymnogyps californianus).* In 1983 there were only four obtainable California Condor chick samples. These were the first ever hatched in captivity. Results were good enough to point to a promising new method, but, there simply were not enough data to verify the technique in California Condors.

It was necessary to plan at least one "proper experiment," with a common species that would be plentiful enough in number to allow a statistical review of results (Bercovitz et al., 1985). The "model bird" continues to be domesticated chickens *(Gallus domesticus).* Their abundance also allowed collection of blood, adrenal and gonad samples for parallel analysis of external and internal hormone data. A second experiment was planned in two endangered species, to evaluate only non-invasive data and to establish a protocol better suited to wider applications.

Two estrogens, estrone (E_1) and estradiol (E_2), have been identified in chicken embryo excrement (Ozon, 1969). Little else was known about perinatal excretory hormones in any bird. Our task was to characterize any sex-related differences measurable in egg waste samples collected at hatching.

Chickens may be the "model birds," but, egg waste estrogen analy-

sis needed to be tested in other species, i.e., in birds of prey, crane or parrot chicks, to verify its utility as a sexing method. Therefore, another experiment was designed (Bercovitz and Sarver, 1988) to compare any sex-related differences of excretory steroids between day-old Andean Condors *(Vultur gryphus)* and Peregrine Falcons *(Falco peregrinus).*

Experimental Methods for Analysis

Egg waste materials consisted of the accumulated allantoic urates and any cloacal expressions during pipping. They were initially ground up in ethanol, dried, mixed with buffer and then hydrolyzed, extracted, purified by liquid chromatography and assayed for estrogens. Essentially they were processed as a normal avian feces sample. Sample preparations were improved by ultrasonically homogenizing the egg wastes directly with buffer (Bercovitz and Sarver, 1988). Various ratios of total or specific estrogenic and androgenic components were surveyed to find non-invasive indicators with the greatest sex difference.

The basic idea behind this work assumed four related concepts: 1) Gonads from normal incubating chicks produced sex steroids, 2) Embryonic ovaries were expected to produce more estrogens than testicular tissue, 3) Steroid metabolites, transferred through the blood, were deposited and accumulated in excretory wastes, 4) Females were expected to produce and excrete higher concentrations of estrogens than male chicks. The comparative aspects, between different species, had not previously been reported for sex differences in excretory steroids. This was the most fascinating aspect of this work.

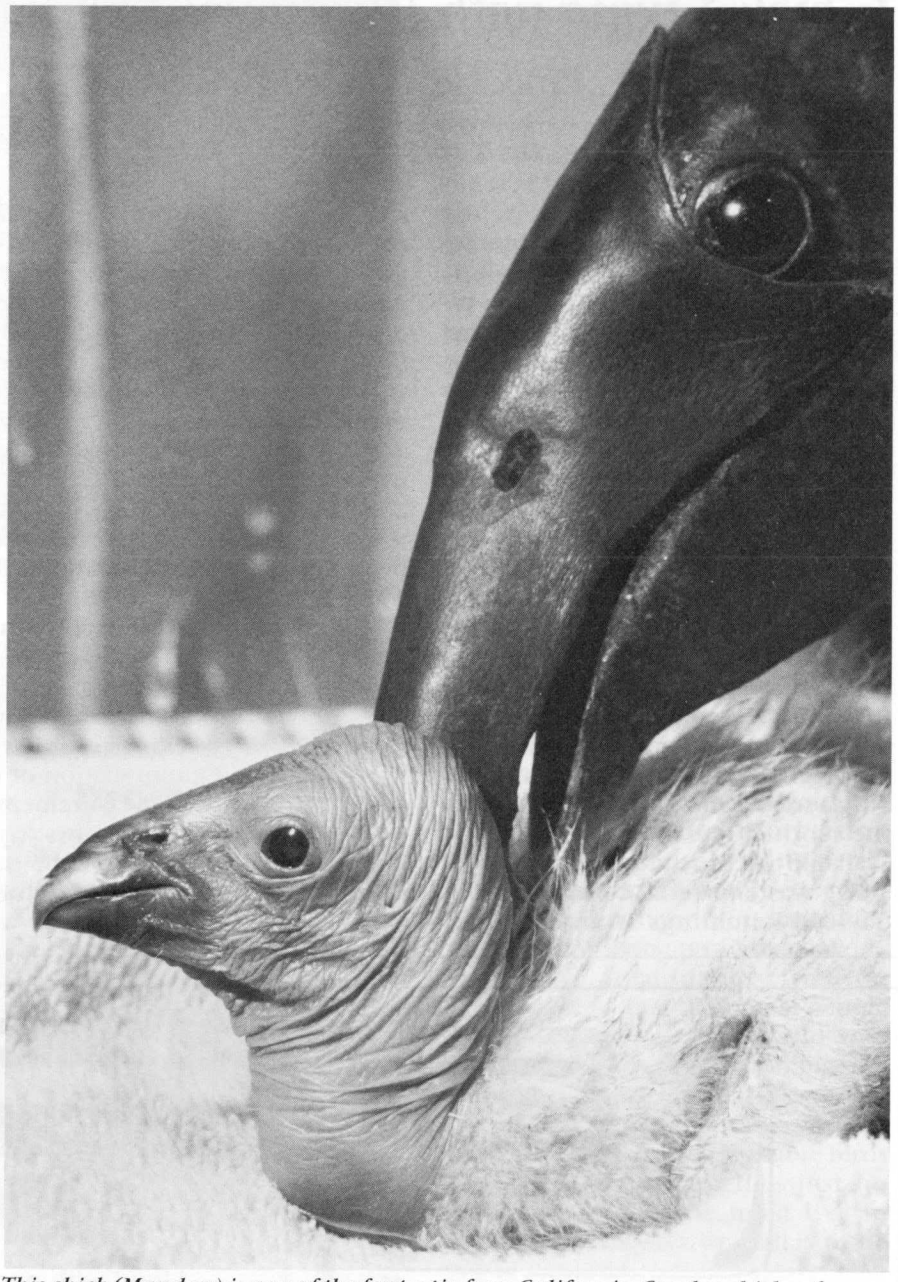
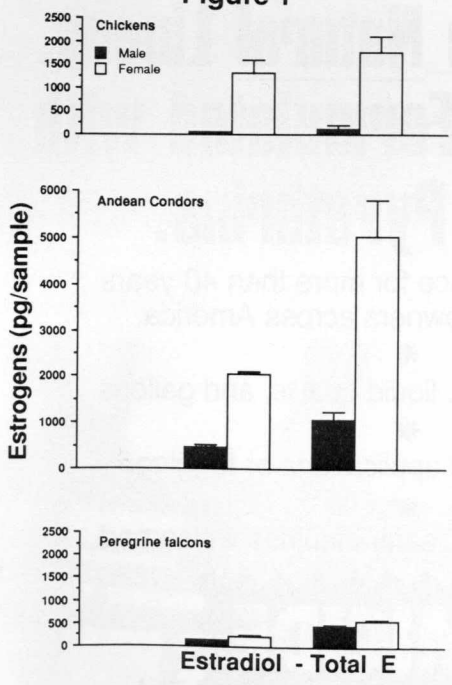


Photo courtesy of Arden Bercovitz, San Diego, CA

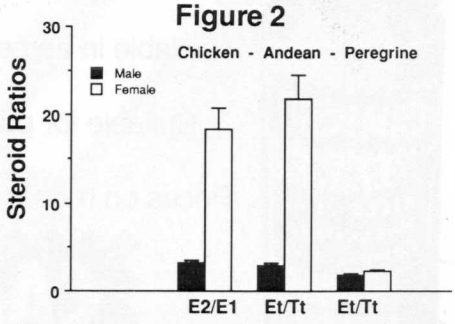
This chick (Mandan) is one of the fantastic four California Condor chicks of 1988-89 shown with the head of adult puppet.

Figure 1



Egg waste estrogen data for estradiol (E_2) and total estrogens (E_t) are illustrated from three species: domesticated chickens (Gallus domesticus), *Andean Condors* (Vultur gryphus) *and Peregrine Falcons* (Falco peregrinus). *Sex-related differences were significant ($p < 0.05$), with all female data (open bars) greater than males (solid bars).*

Figure 2



Sex steroid ratios for excretory estrogens, estradiol:estrone (E_2/E_1) and total estrogen:total testosterone (E_t/T_t) are illustrated from egg waste samples. Sex-related differences are significantly greater in females (open bars) than from males (solid bars) in all three species: domesticated chickens (Gallus domesticus), *Andean Condors* (Vultur gryphus) *and Peregrine Falcons* (Falco peregrinus).

Results and Recommendations

The results were even more successful than expected. Figure 1 illus-

trates excretory estrogen data from chickens (top), Andean Condors (middle) and Peregrine Falcons (bottom). All egg excretory estrogen measurements, E_2 and Total E (E_T), were significantly higher ($p < 0.05$) in females than in males from all three species.

Domesticated chicken E_T data were 19-fold higher and E_2 was 31-fold higher for females than male chickens. Estradiol was the major estrogen in both sexes from excreta, gonad and tissue samples. Sex identity was clearly distinguishable when based on egg waste estrogen data. This material can be collected with complete safety to the chick. Estrogen data from Andean Condors was likewise 2- to 5-fold higher in females than males. Peregrine Falcon data were also significantly different ($p < 0.05$), but female estrogens were only 0.25- to 0.75-fold greater than males.

Figure 2 illustrates excretory sex steroid ratio data for these species. Estrogen components were ratioed to each other or to total androgens. Thus androgens, namely testosterone (Total T) were only measured in condor and falcon samples. Condor males had a 2-fold increase ($p < 0.05$) compared to females, whereas Peregrine androgens did not differ between sexes. All reported measurements were significantly greater ($p < 0.05$) in females than in males: chicken ratios for egg waste E_2/E_1 were nearly 6-fold greater; Andean Condor E_t/T_t ratios were nearly 8-fold greater; Peregrine Falcon E_t/T_t ratios were 1.2-fold greater.

All evaluated measurements were successful in sexing these three species. However, the most fascinating and frustrating aspect of interpreting this data involved discrepancies between species. Current data in these three species did not indicate any common "rule-of-thumb" or single standard applicable to identify males from females. Each species must be evaluated separately. Thus, the expected range of male and female excretory hormone data must be authenticated and validated separately for each species. Data from many more species will be needed before any comparative generalizations or practical applications become possible. This is analogous to a working with a jigsaw puzzle that enlarges at the same time the pieces get smaller. Progress is slower, fitting enough pieces together, to get a recognizable picture.

Conclusions

This research which began in California Condors has now detoured and expanded to include domesticated chickens, Andean Condors and Peregrine Falcons with encouraging results. It will soon come back home to roost. Samples are now being processed from the latest four California Condor chicks hatched during the 1988-89 breeding season. They are the first captive-bred and hatched chicks from this species. The number of California Condors hatched in captivity since 1983 now totals 17.

Final results and verification of egg waste estrogen sexing in this species will soon be completed and ready for scientific publication submission. Extended research with crane and parrot species is also near completion. The close of 1989 will provide a better picture of how this sexing method will work in a wide variety of species and how practical it may be for general use. It will be my pleasure to submit a summary of these findings to *Watchbird* readers as they become completed.

I hope you agree that, through projects such as this, we are indeed closer to fulfilling the goals of the AFA Research Fund. We can encourage conservation through captive breeding, education of the general public and through support of this and other vital scientific research.

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