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Effects of Porcine Follicle-Stimulating Hormone on the Reproductive Performance of Female Zebra Finches (*Taeniopygia guttata*)

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It has been suggested that follicle-stimulating hormone (FSH) may play a role in egg size/number trade-offs in oviparous vertebrates. We tested this hypothesis in an avian species by administering porcine FSH (pFSH) to intact, captive female zebra finches (*Taeniopygia guttata*) during egg formation. We predicted that (1) pFSH would increase the number of ovarian follicles recruited into rapid yolk development and so increase clutch size, (2) an increase in clutch size would lead to a reduction in egg size, and (3) doses of pFSH that were not sufficient to increase clutch size would increase yolk deposition and so increase egg mass. Although a range of pFSH doses decreased egg mass by ca. 10% in three separate experiments, the reduction in egg mass occurred in the absence of an increase in the number of eggs laid. Porcine FSH decreased mean clutch size significantly in one experiment and reduced median clutch size significantly in the other two experiments. The results of this study did not support the hypothesis that FSH mediates a trade-off between egg size and clutch size in birds. © 2002 Elsevier Science (USA)

Key Words: FSH; egg production; egg size; clutch size; trade-off; phenotypic engineering.

INTRODUCTION

A variety of functions such as reproduction and survival compete for limited resources within individual organisms (Stearns, 1992). However, trade-offs will not necessarily be reflected by negative relationships between life-history traits within populations and species if individuals differ in their access to resources (van Noordwijk and de Jong, 1986). Experimental manipulation is therefore required to overcome the confounding effect of individual "quality" and so test for the presence of trade-offs (e.g., Ketterson and Nolan, 1992; Sinervo, 1999).

The theoretical trade-off between propagule size and number has received a great deal of attention, and yet empirical studies based on correlations within species have yielded mixed results (Bernardo, 1996); experimental tests of the egg size/clutch size trade-off have been few (but see Nager *et al.*, 2000). However, Sinervo and Licht (1991) did find experimental evidence of a trade-off between egg size and number in the side-blotched lizard (*Uta stansburiana*): treatment of females with ovine follicle-stimulating hormone increased clutch size by 43% and decreased egg mass by 23%. FSH may therefore play a role in the mechanistic basis of the negative genetic correlation between egg size and clutch size in *U. stansburiana* (Sinervo, 1999).

Since many aspects of reproductive endocrinology are common to all vertebrates (Jones, 1978a; Wallace, 1985), FSH may also underlie a trade-off between egg size and number in other taxa (Sinervo and Licht, 1991). In all vertebrates studied, an increase in the concentration of circulating gonadotropins (e.g., FSH) increases the number of ovarian follicles in more ad-

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vanced stages of development (Jones, 1978a). In nonmammalian vertebrates, FSH also stimulates follicular growth (Jones *et al.*, 1976; Jones, 1978b; Lance and Callard, 1978; see also Tyler *et al.*, 1991) via the uptake of the protein vitellogenin from the circulation (Wallace, 1985). In the domestic hen (*Gallus gallus domesticus*), FSH appears to be involved in the recruitment of new follicles into the ovarian hierarchy, as well as aspects of the maturation and rapid growth of these new follicles (Imai and Nalbandov, 1971; Imai, 1983; Li and Johnson, 1993), although it should be noted that our knowledge of the function of FSH in birds comes largely from studies that examined the effects of mammalian FSH (Etches, 1996). For example, porcine FSH (pFSH) increased the number of ovarian follicles in hens (Palmer and Bahr, 1992). This finding is consistent with the increase in clutch size observed by Sinervo and Licht (1991), although Palmer and Bahr (1992) sacrificed females following treatment and so were not able to report the number of eggs laid subsequently. Lower doses of pFSH increased yolk deposition into the yolky follicles with little change in follicle number (Palmer and Bahr, 1992). Although it is known that circulating FSH levels generally peak during egg formation in wild birds (e.g., Dawson and Goldsmith, 1982; Wingfield and Farner, 1993; Silverin *et al.*, 1997), no study has investigated the effects of exogenous FSH on any aspect of reproductive performance in nondomesticated avian species.

The goal of this study was to examine the effects of exogenous FSH on breeding female birds and to test the hypothesis that FSH mediates a trade-off between egg size and clutch size in an avian species, as has been proposed for lizards (Sinervo and Licht; 1991, Sinervo, 1999). We administered pFSH to intact, captive, female zebra finches (*Taeniopygia guttata*) during egg formation and studied its effects on primary reproductive effort (clutch size and egg mass and composition). We predicted that (1) pFSH would increase the number of ovarian follicles recruited into rapid yolk development and so increase the number of eggs laid (as in Sinervo and Licht, 1991) and (2) an increase in the number of growing follicles (Prediction 1) would increase the competition between follicles for circulating vitellogenin and so result in smaller yolks and eggs (as in Sinervo and Licht, 1991). Since competition between follicles for vitellogenin is the hypothesized mechanism behind the proposed egg size/number trade-off (Prediction 2), we measured the circulating levels of this yolk precursor. In addition, we predicted that (3) doses of pFSH that were not sufficient to increase clutch size would increase yolk deposition (as in Palmer and Bahr, 1992; see also Follett *et al.*, 1968; Tyler *et al.*, 1991) and so increase yolk and egg mass.

MATERIALS AND METHODS

General

The experimental protocol followed the guidelines of the Canadian Council on Animal Care (Simon Fraser University Animal Care Committee Projects 526B, 558B). A captive-breeding population of zebra finches was maintained under controlled environmental conditions (temperature 24–28°C; humidity 35–55%; constant light schedule, 14 L:10 D, lights on at 07:00), with birds kept in single-sex cages prior to experiments. All birds were provided with mixed seed (white and panicum millet: 11.7% protein, 0.6% lipid, and 84.3% carbohydrate), water, grit, and cuttlefish bone *ad libitum* and received a multivitamin supplement in the drinking water once per week. During breeding, birds also received a daily egg-food supplement (20.3% protein, 6.6% lipid). Breeding pairs were housed individually in cages (51 × 39 × 43 cm), each with an external nest box (14 × 14 × 20 cm). Females were introduced to the breeding cages in the morning and a single male partner was added later that day. Birds were assigned to experimental groups and pairs at random. Nest boxes were checked daily and all new eggs were weighed (±0.001 g) and numbered. "Laying interval" was defined as the number of days between pairing and the day the first egg was laid (including the day of pairing).

Eggs are generally laid at daily intervals, but females occasionally "skip" a day, i.e., lay two eggs ca. 48 h apart (Williams, 1996). "Clutch size" was therefore defined as the number of eggs laid, allowing interruptions of 1 day during laying. In unmanipulated birds, skipping generally occurs near the end of the clutch (personal observation), and so we noted not only the proportion of birds that skipped during laying, but also the proportion that skipped "early," i.e., within the first three eggs.

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TABLE 1
Summary of Experimental Protocols

Experiment	FSH dose/injection (μg pFSH/g body mass)	Number of injections	Vehicle	Sample size per group	Collected female at clutch completion?
1	0, 0.0005, 0.0080, 0.3125	5	PBS	10	No
2	0, 0.3125	5	PBS + 0.5% BSA	10	Yes
3	0, 0.0313, 0.0625	Daily until one-egg stage	PBS + 0.5% BSA	13	No

Females were weighed (±0.1 g) at the time of pairing, on the day the first egg was laid (i.e., one-egg stage), and again 2 days after the last egg was laid (i.e., clutch completion). Females were also blood sampled at the one-egg stage for measurement of plasma vitellogenin levels. Females that failed to lay eggs within 13 days of pairing were returned to the single-sex cages. This study consisted of three experiments that are described below and summarized in Table 1.

Hormone Treatment

Porcine FSH was used since it had been shown to have physiological effects in birds in other studies (e.g., Palmer and Bahr, 1992) and since it was available in larger quantities than chicken FSH. In the absence of data regarding the effects of pFSH on small passerines, the studies of Sinervo and Licht (1991) and Palmer and Bahr (1992) were used as starting points when selecting dosages (see below). However, it should be noted that the effective dosage of a hormone will depend on a number of factors, including mass-specific metabolic rate, which undoubtedly varies greatly between side-botched lizards, chickens, and zebra finches. Taxonomic differences between the source of the hormone and the study species further complicate matters, and thus we do not attempt to directly compare results at different dosages between studies.

Experiment 1

Experiment 1 was designed to test a range of doses bracketing those used by Palmer and Bahr (1992) (0.01–0.22 μg/g body mass per day) and by Sinervo and Licht (1991) (i.e., 2.5 μg/g every other day or 1.25 μg/g per day). The activity of the pFSH preparation we obtained from Dr. J. Proudman of the USDA Animal Hormone Program (USDA-pFSH-I-1) was roughly 40 times more biologically potent than that used by Palmer and Bahr (1992) (USDA-pFSH-B1) and 4 times more potent than that used by Sinervo and Licht (1991) (NIH-FSH-S16) (John Proudman, personal communication). We therefore used the following doses: 0.0005, 0.0080, and 0.3125 μg pFSH/g body mass in 100 μl phosphate-buffered saline (PBS), assuming an average mass of 17 g (Williams, 1996). These doses were administered daily on 5 consecutive days, starting on the day of pairing, by intraperitoneal injection (between 10:45 and 13:45). A control group received five daily doses of 100 μl PBS. Ten females were assigned to each treatment. A preliminary experiment revealed that intraperitoneal injections during egg formation and blood sampling at the one-egg stage did not affect egg production (proportion of females that laid eggs, time between pairing and egg-laying, clutch size, and mean egg mass; data not shown).

Experiment 2

To confirm the effects of the high dose observed in Experiment 1 (see Results), we repeated this experiment using only two treatments: the 0.3125 μg pFSH/g dose and controls ($N = 10$ per treatment). To examine the possibility that pFSH increased the number of follicles recruited into rapid yolk development, but that not all growing follicles were ovulated, we assessed the ovarian state of the females at clutch completion (i.e., 2 days after the last egg was laid). Females were killed by exsanguination from the jugular vein under anesthesia (mixture of ketamine and xylazine at doses of 20 and 4 mg/kg, respectively), and the ovary was dissected from the carcass and weighed (±0.001 g).

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For Experiments 2 and 3, a new batch of pFSH-I-1 (lot AFP-10640B) of the same biopotency as that used in Experiment 1 was obtained from Dr. A. F. Parlow of the U.S. NIDDK National Hormone and Pituitary Program. The level of contamination of this preparation with other anterior pituitary hormones was 1.5% for luteinizing hormone and less than 1% for thyroid-stimulating hormone, prolactin, and growth hormone combined (A. F. Parlow, personal communication). In Experiments 2 and 3, 0.5% bovine serum albumin fraction V was added to the PBS to reduce the absorption of pFSH to the container.

Experiment 3

In Experiment 1, only the highest dose (0.3125 μg/g) had significant effects on egg production (see Results). However, this dose was approximately 40 times greater than the next highest dose (0.0080 μg/g), and so in Experiment 3 we investigated the effects of moderately lower doses (10 and 20% of the highest dose). Three treatments were used in this experiment: 0, 0.0313, and 0.0625 μg pFSH/g ($N = 13$ per treatment). Because of the reduced dosages, we were not limited to five injections per bird. We therefore administered daily doses from the day of pairing until the day the first egg was laid (inclusive).

Measurement of Plasma Vitellogenin Concentrations

Plasma levels of vitellogenin were measured using the vitellogenic zinc method developed for the domestic hen (Mitchell and Carlisle, 1991) and validated for passerines (Williams and Martyniuk, 2000). This method measures the concentration of zinc bound to vitellogenin (i.e., vitellogenic zinc) as an index of the plasma concentration this protein (Mitchell and Carlisle, 1991). Vitellogenic zinc levels are obtained from the difference between zinc in unmanipulated plasma (i.e., total zinc—Wako Chemicals) and that in plasma that has been depleted of vitellogenin by precipitation with dextran sulfate (i.e., depleted zinc). For some samples, plasma volume was too small to allow measurement of depleted zinc, so only total zinc was assayed. Vitellogenic zinc in these samples was calculated from the regression of vitellogenic zinc on total zinc from samples for which we had both total and depleted zinc values. The interassay coefficient of variation for the assays performed in this study was 7.6% ($N = 8$).

Egg Composition

To determine the macronutrient composition of the eggs, the first and third eggs were collected on the day they were laid and replaced with eggs laid by unmanipulated females. These eggs were boiled for 10 min, frozen, and later separated into shell, albumen, and yolk. All components were dried to constant mass in a drying oven at 55°C. Lipids were extracted from yolks by Soxhlet extraction for 8 h, with petroleum ether as the solvent (Dobush *et al.*, 1985). Yolk lipid was calculated from the difference between dry yolk mass and lean dry yolk mass. Dry albumen and lean dry yolk are approximately 88% protein (Burley and Vadehra, 1989) and were used as measures of albumen and yolk protein, respectively. For each egg component, the means of the first and third egg are presented (although for some samples data were available for only one egg). A number of eggs were damaged during analysis (i.e., cracked during boiling) and so sample sizes are reduced slightly for these parameters.

Statistics

All statistical analyses were carried out using SAS (SAS Institute, 1989). Parametric tests were performed for most variables (general linear model wherever F values are provided; proc GLM; SAS Institute, 1989), but rank-order statistics were used to analyze clutch size and laying interval (Wilcoxon two-sample test for comparing two groups (Z values are provided), Kruskal–Wallis test for comparing more than two groups (χ^2 values), median one-way analysis for comparing medians (χ^2 values); proc NPAR1WAY; SAS Institute, 1989). In analyses of mean egg mass, egg composition, and female mass, we controlled for the mass of the female at pairing (i.e., pretreatment) by including this term as a covariate in the model. Egg mass is highly repeatable within individual females (Williams, 1996) and so we also included egg mass from a previous, unmanipulated clutch as a covariate in analyses of mean egg mass to control for interindividual variation in egg mass. Where a significant effect of treatment was found ($P < 0.05$), treatment means were compared with controls using contrasts (Sokal

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TABLE 2
Effects of pFSH on Reproductive Parameters in Experiment 1

	Control	0.0005 μg/g	0.0080 μg/g	0.3125 μg/g
Proportion of females that laid eggs	100% (10/10)	90% (9/10)	90% (9/10)	90% (9/10)
Laying interval (days)	6.0 ± 0.6	4.9 ± 0.7	5.8 ± 0.7	6.0 ± 0.7
Clutch size	5.2 ± 0.5	5.9 ± 0.5	4.1 ± 0.5	3.2 ± 0.5*
Proportion of females that skipped 1 or more days during laying	30% (3/10)	33% (3/9)	44% (4/9)	33% (3/9)
Proportion of females that skipped a day within the first three eggs	20% (2/10)	22% (2/9)	44% (4/9)	33% (3/9)
Mean egg mass (g)[a]	1.06 ± 0.03	1.07 ± 0.04	1.06 ± 0.03	0.94 ± 0.03*
Mean yolk lipid (mg)[b]	63 ± 3	64 ± 3	62 ± 3	59 ± 3
Mean yolk protein (mg)[b]	39 ± 2	37 ± 2	35 ± 2	33 ± 2
Mean albumen protein (mg)[b]	76 ± 3	79 ± 4	78 ± 4	70 ± 4
Mean dry shell mass (mg)[b]	60 ± 2	60 ± 2	60 ± 2	56 ± 2
Plasma vitellogenin (μg/ml vitellogenic zinc)	2.6 ± 0.3	2.0 ± 0.3	2.0 ± 0.4	1.5 ± 0.3

Note. Values are least-squares means ± standard errors.
[a] Controlling for egg mass from a previous breeding attempt and female mass at pairing.
[b] Controlling for female mass at pairing.
* $P < 0.05$.

and Rohlf, 1995; pdiff option; SAS Institute, 1989). For nonparametric tests, multiple comparisons were performed by comparing each treatment with controls separately. For all multiple comparisons, Bonferroni-adjusted α levels were used (i.e., in Experiment 1, α = 0.05/3 pFSH treatments = 0.017; in Experiment 3, α = 0.05/2 pFSH treatments = 0.025); we compared pFSH-treated groups only with controls (and not with each other) to reduce the number of comparisons. χ^2 tests were used to compare frequencies between groups. Values are presented as least-squares means ± standard errors (SAS Institute, 1989).

RESULTS

Experiment 1

Treatment with pFSH did not affect the probability of laying eggs ($\chi^2_3 = 1.08$, $P > 0.2$; Table 2) or the time taken to initiate laying ($\chi^2_3 = 1.59$, $P > 0.2$; Table 2). There was a significant effect of treatment on clutch size (Kruskal–Wallis, $\chi^2_3 = 13.36$, $P < 0.01$; median, $\chi^2_3 = 12.59$, $P < 0.01$; Table 2, Fig. 1A), with the females in the 0.3125 μg/g treatment laying, on average, two eggs fewer than controls ($Z = -2.50$, $P = 0.0126$). Treatment with the 0.0005 and 0.0080 μg/g doses did

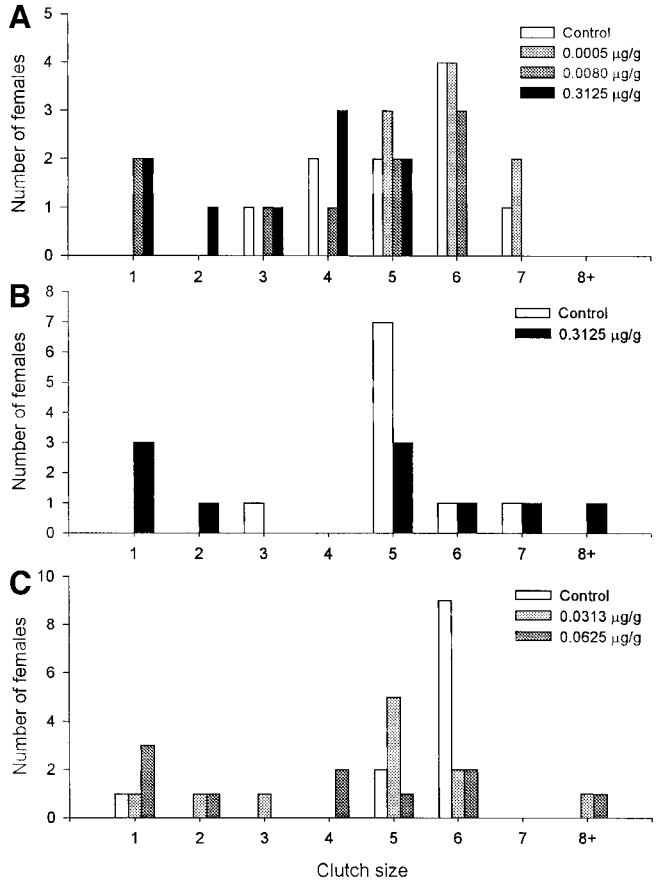

FIG. 1. Frequency distribution of clutch sizes in (A) Experiment 1, (B) Experiment 2, and (C) Experiment 3.

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TABLE 3
Effects of pFSH on Reproductive Parameters in Experiment 2

	Control	0.3125 μg/g
Proportion of females that laid eggs	100% (10/10)	100% (10/10)
Laying interval (days)	6.4 ± 0.4	7.3 ± 0.4
Clutch size	5.7 ± 0.7	4.2 ± 0.7*[a]
Proportion of females that skipped a day within the first three eggs	10% (1/10)	30% (3/10)
Mean egg mass (g)[b]	1.09 ± 0.02	0.99 ± 0.02*
Mean yolk lipid (mg)[c]	67 ± 3	56 ± 3*
Mean yolk protein (mg)[c]	42 ± 2	32 ± 2**
Mean albumen protein (mg)[c]	80 ± 3	70 ± 3*
Mean dry shell mass (mg)[c]	62 ± 2	54 ± 2**
Ovary mass at clutch completion (mg)[d]	41 ± 4	39 ± 5
Plasma vitellogenin (μg/ml vitellogenic zinc)	2.2 ± 0.3	2.3 ± 0.3

Note. Values are least-squares means ± standard errors.
[a] Significant difference in median clutch size.
[b] Controlling for egg mass from a previous breeding attempt and female mass at pairing.
[c] Controlling for female mass at pairing.
[d] Controlling for nonreproductive mass of female.
* $P < 0.05$.
** $P < 0.01$.

not affect clutch size ($P > 0.2$ for all comparisons with controls). There was no effect of pFSH on the proportion of females that skipped one or more days during laying ($\chi^2_3 = 0.48$, $P > 0.2$; Table 2) or that skipped within the first three eggs ($\chi^2_3 = 1.68$, $P > 0.2$; Table 2).

The effect of pFSH on mean egg mass was significant ($F_{3,25} = 3.08$, $P = 0.05$; controlling for egg mass from a previous breeding attempt and female mass at pairing; Table 2). Treatment with 0.3125 μg/g reduced egg mass by 11%, compared with controls ($P = 0.0125$). Mean egg mass in the other treatments did not differ from that in controls ($P > 0.2$ for comparisons). Although the mean composition of the first and third eggs (controlling for female mass at pairing) did not differ between treatments (yolk lipid, $F_{3,28} = 0.54$, $P > 0.2$; yolk protein, $F_{3,28} = 1.42$, $P > 0.2$; albumen protein, $F_{3,26} = 0.84$, $P > 0.2$; dry shell, $F_{3,27} = 0.68$, $P > 0.2$; Table 2), all components were lightest in the 0.3125 μg/g group (Table 2).

The effect of treatment on the plasma concentrations of vitellogenin was not significant ($F_{3,26} = 1.99$, $P > 0.1$; Table 2). Female mass did not differ between treatments at the one-egg stage ($F_{3,28} = 1.80$, $P > 0.15$; Table 2) or at clutch completion ($F_{3,26} = 1.07$, $P > 0.2$; Table 2), controlling for female mass at pairing.

Experiment 2

All females produced eggs. As in Experiment 1, treatment with 0.3125 μg pFSH/g did not affect laying interval ($Z = 1.21$, $P > 0.2$; Table 3). The effect of the 0.3125 μg/g dose on clutch size was similar to that observed in Experiment 1, with pFSH-treated females laying 1.5 fewer eggs than controls, on average (Table 3; Fig. 1B). Although the difference in ranks was not significant ($Z = -1.57$, $P > 0.1$), median clutch size did differ between groups ($\chi^2_1 = 4.16$, $P < 0.05$). The proportion of females that skipped a day during laying did not differ between treatments ($\chi^2_1 = 1.25$, $P > 0.2$; Table 3); all of the females that skipped a day during laying did so within the first three eggs.

Mean egg mass was again reduced by the 0.3125 μg/g dose ($F_{1,16} = 8.41$, $P < 0.05$; controlling for egg mass from a previous breeding attempt and female mass at pairing; Table 3), in this case by 9%. In this experiment the effect on egg mass was reflected in the composition of the first and third eggs (controlling for female mass at pairing; Table 3). Yolk lipid was decreased by 16% ($F_{1,17} = 7.36$, $P < 0.05$), yolk protein by 24% ($F_{1,17} = 18.16$, $P < 0.01$), albumen protein by 13% ($F_{1,15} = 5.25$, $P < 0.05$), and dry shell by 13% ($F_{1,15} = 10.45$, $P < 0.01$).

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TABLE 4
Effects of pFSH on Reproductive Parameters in Experiment 3

	Control	0.0313 μg/g	0.0625 μg/g
Proportion of females that laid eggs	92% (12/13)	85% (11/13)	77% (10/13)
Laying interval (days)	6.0 ± 0.8	5.0 ± 0.8	6.4 ± 0.9
Clutch size	5.4 ± 0.6	4.6 ± 0.6[a]	3.8 ± 0.6[a]
Proportion of females that skipped 1 or more days during laying	33% (4/12)	27% (3/11)	50% (5/10)
Proportion of females that skipped a day within the first three eggs	0% (0/12)	18% (2/11)	50% (5/10)*
Mean egg mass (g)[b]	1.10 ± 0.03	1.00 ± 0.03*	0.98 ± 0.03*
Mean yolk lipid (mg)[c]	66 ± 2	53 ± 2*	58 ± 3
Mean yolk protein (mg)[c]	42 ± 2	32 ± 2*	35 ± 2
Mean albumen protein (mg)[c]	79 ± 3	72 ± 4	68 ± 4
Mean dry shell mass (mg)[c]	60 ± 2	52 ± 2*	52 ± 2*
Plasma vitellogenin (μg/ml vitellogenic zinc)	2.3 ± 0.3	2.5 ± 0.3	2.2 ± 0.3

Note. Values are least-squares means ± standard errors.
[a] Significant variation in median clutch size; marginally significant in paired comparisons.
[b] Controlling for egg mass from a previous breeding attempt and female mass at pairing.
[c] Controlling for female mass at pairing.
* $P < 0.05$.

Treatment with pFSH did not affect the wet masses of the ovary on the second consecutive day that no egg was laid ($F_{1,14} = 0.13$, $P > 0.2$), controlling for nonreproductive mass (i.e., the mass of the female at collection minus the wet masses of the ovary and oviduct). Three pFSH-treated females had an egg in their oviduct and therefore would have laid an egg after skipping 2 days; these birds were excluded from the analysis of ovary mass. Although no control females had oviducal eggs in this experiment, 1 of 10 control females in Experiment 1 and 2 of 12 control females in Experiment 3 did lay eggs after skipping 2 days.

Treatment with pFSH did not affect the plasma concentrations of vitellogenin ($F_{1,17} = 0.02$, $P > 0.2$); the mean values for treated females and controls were very similar (Table 3). Female mass did not differ between groups at the one-egg stage ($F_{1,17} = 0.01$, $P > 0.2$; Table 3) or at clutch completion ($F_{1,17} = 1.85$, P > 0.15; Table 3), controlling for female mass at pairing.

Experiment 3

Treatment with pFSH did not affect the probability of laying eggs ($\chi^2_2 = 1.18$, $P > 0.2$; Table 4) or the time taken to initiate laying ($\chi^2_2 = 2.24$, $P > 0.2$; Table 4). Females treated with 0.0313 and 0.0625 μg pFSH/g laid on average 0.8 and 1.6 eggs fewer than controls, respectively (Table 4; Fig. 1C). As in Experiment 2, the ranks of clutch sizes did not differ between treatments ($\chi^2_2 = 4.66$, $P = 0.10$; Table 4), but median clutch sizes did ($\chi^2_2 = 6.71$, $P < 0.05$). In comparisons with controls, the effect of treatment on median clutch size was marginally nonsignificant for both 0.0313 μg/g females ($\chi^2_1 = 4.74$, $P = 0.03$) and 0.0625 μg/g females ($\chi^2_1 = 3.99$, $P = 0.05$), using a Bonferroni-adjusted α of 0.025. The proportion of females that skipped a day during laying did not differ between treatments ($\chi^2_2 = 1.24$, $P > 0.2$; Table 4). However, treatment did affect the frequency of skipping within the first 3 eggs ($\chi^2_2 = 8.25$, $P < 0.05$; Table 4); half of the females in the 0.0625 μg/g group skipped early in the clutch (comparison with controls: $\chi^2_1 = 7.76$, $P < 0.01$).

As in the previous experiments, there was an effect of pFSH on mean egg mass ($F_{2,25} = 4.88$, $P < 0.05$; controlling for egg mass from a previous breeding attempt and female mass at pairing; Table 4). Egg mass was reduced by 9 and 11% in the 0.0313 ($P = 0.02$) and 0.0625 μg/g treatments ($P = 0.01$), respectively (Table 4). These differences were reflected in the yolk components of the first and third eggs (controlling for female mass at pairing; Table 4). Yolk lipid differed among treatments ($F_{2,26} = 7.46$, $P < 0.01$) and was reduced in both the 0.0313 ($P < 0.01$) and the 0.0625 μg/g group ($P = 0.03$), although the latter

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comparison was marginally nonsignificant at $\alpha = 0.025$. Similar results were observed for yolk protein (overall effect of treatment, $F_{2,26} = 5.00$, $P < 0.05$; control vs 0.0313 μg/g, $P < 0.01$; control vs 0.0625 μg/g, $P = 0.06$). Dry shell mass also differed between treatments ($F_{2,24} = 6.36$, $P < 0.01$) and was decreased by both pFSH treatments ($P < 0.01$ in both comparisons). However, the effect of treatment on albumen protein was not significant ($F_{2,24} = 2.33$, $P > 0.1$).

As in Experiment 2, plasma concentrations of vitellogenin differed little between treatments ($F_{2,30} = 0.38$, $P > 0.2$; Table 4). Variation among treatments in the mass of the female at the one-egg stage was marginally nonsignificant ($F_{2,29} = 3.02$, $P = 0.06$, controlling for mass at pairing; Table 4), with the 0.0313 μg/g females being slightly heavier (17.4 ± 0.3 g) than those in the other groups (control, 16.6 ± 0.3 g; 0.0625 μg/g, 16.5 ± 0.3 g). There was no effect of treatment on female mass at clutch completion ($F_{2,29} = 1.63$, $P > 0.2$, controlling for mass at pairing; Table 4).

Relationship between Egg Mass and Clutch Size

To investigate the relationship between egg mass and clutch size, we pooled data from all experiments into three groups: controls (including the 0.0005 and 0.0080 μg/g treatments, which did not differ from controls), medium pFSH (0.0313 and 0.0625 μg/g treatments), and high pFSH (0.3125 μg/g). In a general linear model with mean egg mass as the dependent variable and clutch size and treatment as the main effects, the clutch size × treatment interaction term was marginally nonsignificant ($F_{2,77} = 2.49$, $P = 0.09$), suggesting that the relationship between egg mass and clutch size differed between treatments (Fig. 2). The regression of egg mass on clutch size was not significant in controls ($r^2 = 0.01$, $F_{1,43} = 0.29$, $P > 0.2$), but was positive and significant in the medium pFSH ($r^2 = 0.37$, $F_{1,17} = 9.83$, $P < 0.01$) and high pFSH ($r^2 = 0.34$, $F_{1,17} = 8.68$, $P < 0.01$) groups.

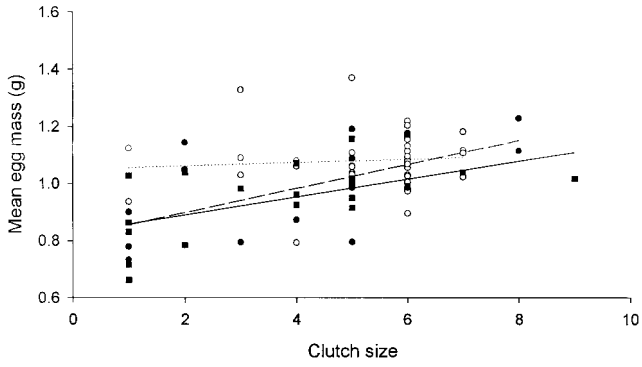

FIG. 2. Effect of pFSH on the relationship between mean egg mass and clutch size in the control (open circles, dotted line), medium pFSH (closed circles, dashed line), and high pFSH (closed squares, solid line) groups.

DISCUSSION

Sinervo (1999) proposed that the trade-off between egg size and clutch size arises from variation in the production of FSH and/or the sensitivity of target tissues to this hormone in the lizard *U. stansburiana.* This mechanism may also underlie egg size/number trade-offs in other groups of vertebrates (Sinervo and Licht, 1991). The primary objective of this study was to investigate this hypothesis in an avian species. A related goal was to determine whether FSH could be used for phenotypic engineering (sensu Ketterson and Nolan, 1992), i.e., to manipulate the number and size of eggs produced and so elucidate the costs of egg production.

In contrast to our third prediction, pFSH did not increase egg mass at any of the doses examined (cf. Palmer and Bahr, 1992). Rather, the medium and high doses of pFSH used in this study (0.0313–0.3125 μg/g) consistently decreased egg mass by ca. 10% in three separate experiments. However, this decrease in egg mass was not associated with an increase in clutch size, in contrast to our first and second predictions. Females treated with medium and high doses of pFSH consistently laid fewer eggs than controls, although only the change in median clutch size was significant in the latter two experiments. The relationship between the number and the mass of eggs was positive in treated females but not in controls (Fig. 2), contrary to our expectation that treatment with pFSH would reveal a trade-off (i.e., negative relationship) between these two parameters.

Effect of pFSH on Clutch Size

The effect of pFSH on clutch size was opposite to our first prediction; treatment tended to decrease

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clutch size. A possible explanation for this result is that exogenous FSH had a negative feedback effect, reducing the endogenous secretion of FSH. In lizards, doses of FSH higher than those used by Sinervo and Licht (1991) have been found to have pharmacological effects, such as blocking ovulation (Licht, 1970). However, it is unlikely that pFSH blocked ovulation in our experiments. Exogenous FSH did not reduce the proportion of females that laid eggs or the number of days between pairing and the day the first egg was laid. Thus, the ovulation of the first egg was not prevented, even though pFSH levels would have been higher at this time than at subsequent ovulations (treatment generally ended on or before the day the first egg was laid). The increased rate of skipping early during laying may reflect pharmacological effects of pFSH, but this effect was significant only at a medium dose (Experiment 3) and not at higher doses. Therefore, pFSH probably reduced the recruitment of follicles, rather than interfering with their ovulation.

Exogenous FSH may have failed to increase clutch size in these experiments because of the timing of treatment. Haywood (1993) suggested that clutch size in the zebra finch is generally determined on the third day of laying, with the smaller follicles in the ovarian hierarchy ceasing growth and failing to proceed to ovulation. Treatment with pFSH generally ended at least 2 days before this "decision" was made. It is possible that prolonged treatment with pFSH throughout laying would have stimulated smaller follicles to continue growing and thus have increased clutch size. Although the timing of treatment could explain why pFSH did not increase clutch size, it does not explain why clutch size was reduced; negative feedback on endogenous FSH secretion remains the most likely explanation for this result.

Effect of pFSH on Egg Mass

The medium and high doses of pFSH used in this experiment consistently reduced egg mass. Although we expected a decrease in egg mass associated with an increase in clutch size, in the absence of an increase in clutch size, we predicted that pFSH would increase egg mass (Prediction 3) due to its role in follicular growth and uptake of yolk proteins (Jones, 1978b). Palmer and Bahr (1992) reported that low doses of pFSH increased yolk deposition into the yolky follicles of chickens. Similarly, in the South African clawed toad (*Xenopus laevis*) Follett *et al.* (1968) found that FSH and estradiol increased the accumulation of radioactive vitellogenin by the ovaries, whereas estradiol alone decreased precursor uptake. In maturing rainbow trout, *Oncorhynchus mykiss*, gonadotropin I (which may be homologous to FSH) increased the rate of vitellogenin uptake into oocytes both *in vivo* and *in vitro* (Tyler *et al.*, 1991; see also Tyler *et al.*, 1997). The reduction in egg mass by pFSH observed in this study may have been due to a reduction in endogenous FSH secretion as a result of negative feedback.

An alternative explanation for the reduction in egg mass is that pFSH did increase the number of growing follicles (and hence competition between follicles for circulating yolk proteins and lipids), but somehow prevented all of the follicles from ovulating. However, as discussed above, it is unlikely that pFSH interfered with ovulation. Furthermore, in Experiment 2 we found no evidence that pFSH increased the number of growing follicles; treated females collected 2 days after clutch completion did not have heavier ovaries than controls. Finally, if pFSH reduced egg mass via an increase in the number of growing follicles and hence competition for yolk precursors, it would be expected that circulating vitellogenin concentrations would be lower in treated females; this was not observed. Negative feedback on pituitary FSH secretion, and not increased competition between follicles for yolk proteins and lipids, is the most probable explanation for the reduction in egg mass.

Egg Size/Number Trade-Off

Our results contrast with those of Sinervo and Licht (1991), who found that exogenous FSH revealed a trade-off between egg size and number by increasing clutch size and decreasing egg mass in *U. stansburiana.* This discrepancy may result from the difference in the pattern of ovulation between *U. stansburiana* and birds: in *Utas*, all follicles are yolked and ovulated simultaneously (Licht, 1970), whereas in birds the growth of follicles occurs in a hierarchy and follicles are ovulated sequentially (at most one per day). These patterns could lead to a fundamental difference in the nature of the egg size/number trade-off. In vertebrates that produce multiple eggs simultaneously, an increase in clutch size requires an increase in the num-

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ber of growing ovarian follicles and hence an increase in competition between follicles for circulating yolk precursors. In contrast, birds can increase their clutch size without increasing the number of growing follicles in the ovary at any one time. Instead, producing more eggs requires females to continue laying for a longer period of time. Thus, the trade-off between egg mass and clutch size may be reduced in birds. Consistent with this suggestion, a radiotracer study of protein deposition into yolks found no evidence of competition between follicles for yolk precursors in the zebra finch (Christians and Williams, 2001). However, in species that rely on endogenous nutrient stores (see Meijer and Drent, 1999), females must allocate a fixed amount of nutrients among eggs, and thus a trade-off may still be present (e.g., Nager *et al.*, 2000). Birds may also face trade-offs between the benefits of producing more eggs and the costs of increasing the duration of egg production (e.g., increased susceptibility to predation; Lee *et al.*, 1996).

Despite the potential complications with the administration of pFSH in our experiments (negative feedback, timing of treatment), this study clearly shows that pFSH may decrease egg mass in the absence of an increase in clutch size. Thus, our data do not support the hypothesis that FSH underlies an egg size / number trade-off in birds. We suggest that the mechanistic basis of this trade-off differs between birds and some other vertebrates as a result of the difference in the pattern of ovulation (i.e., sequential vs simultaneous) between these groups.

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