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Analysis of Poultry Fertility Data. 2. Comparison of Long- and Short-Term Fertility Trials[1,2]

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ABSTRACT   The purpose of the present study was to determine the effect of egg collection interval on interpretation of fertility trial results. Low-fertility (LF) and randombred (RB) Delaware hens were inseminated with spermatozoa from LF or RB Delaware roosters. Three replicate trials were performed for each cross. Eggs were collected for 21 days following insemination. Fertility was analyzed with a log odds model following logit transformation. Separate analyses were performed on data collected throughout the first 7 days and the entire egg collection interval. Duration of fertility over the 21-day interval was analyzed by iterative least squares. Whereas the 21-day egg collection interval afforded detection of differences between males and between females, no differences were observed following analysis of data from the 7-day interval. Differences in reproductive efficiency also were detected when data were analyzed by iterative least squares. Thus, the value of a fertility trial is, in part, determined by the length of the egg collection interval.

(*Key words*: data analysis, duration of fertility, fertility, logits, chickens)

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INTRODUCTION

The ultimate test of reproductive efficiency is the fertility trial. However, commonly used experimental schemes and statistical methods may limit the utility of a fertility trial. For example, when artificial insemination is used, chickens and turkeys are routinely inseminated weekly and biweekly, respectively (Sexton, 1983). Although these insemination frequencies may yield optimal fertility, they also preclude estimation of useful parameters (Kirby and Froman, 1990).

Fertility trials have been instrumental in characterizing heritable male subfertility in the Delaware breed (Froman and Bernier, 1987; Kirby et al., 1989, 1990; Froman and Engel, unpublished data). Inferences made in these studies were dependent upon patterns of fertility. This approach has also been useful in the study of subfertility associated with homozygosity for the rose comb allele (Kirby et al., 1989; Froman et al., unpublished data) and subfertility induced by alteration of the spermatozoon's glycocalyx (Froman and Engel, 1989). Thus, duration of fertility is a parameter highly suited for assessing spermatozoal function within the oviduct.

However, duration of fertility is a variable of practical interest as well because any reduction in artificial insemination frequency without loss of fertility would reduce the cost of producing hatching eggs. Although duration of fertility may be predicted from data obtained from egg collection intervals of 18 to 21 days (Kirby and Froman, 1990), such an interval may seem impractical, especially if overall fertility is the only variable of interest. Therefore, the purpose of the present study was to determine the effect of egg collection interval on the interpretation of fertility trial results.

MATERIALS AND METHODS

For the purpose of comparison, fertility data were obtained from three replicate fertility trials in which Delaware hens from randombred[5] (RB) and low-fertility (LF) lines were inseminated with spermatozoa from either RB or LF roosters. Males within the LF line were

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characterized by spermatozoal degeneration as described previously (Froman and Bernier, 1987; Froman *et al.*, 1990). Low-fertility males were ejaculated daily for 4 days prior to each fertility trial in order to maximize the percentage of viable spermatozoa in ejaculates (Froman and Bernier, 1987).

In each trial, semen was collected from each of five RB and five LF roosters by abdominal massage (Burrows and Quinn, 1937). Ejaculates were pooled according to male line. Spermatozoal concentration and percentage of viable spermatozoa were determined according to Bilgili and Renden (1984). Pooled semen was diluted to 2×10^9 viable spermatozoa per milliliter with Beltsville Poultry Semen Extender,[6] pH 7.5. Both RB and LF hens (8 to 12 per line) were inseminated with extended semen from each male line. Each hen was inseminated with 1×10^8 viable spermatozoa in a volume of 50 μL.

Eggs were collected throughout a 21-day interval, which commenced on the 2nd day following insemination. Eggs were identified by hen and date, set at weekly intervals, and broken open after 4 days of incubation. Fertility was assessed by examining contents for embryonic development. Percentages of fertilized eggs were calculated per hen for the first 7 days of egg collection as well as the entire 21-day interval.

Percentages derived from the 21-day intervals were transformed to logits according to Kirby and Froman (1990). Each logit, L_i, was calculated as follows:

$$L_i = \log_e[(r_i - .5) \div (n_i - r_i - .5)],$$

where r_i and n_i denoted the number of fertilized and total eggs, respectively, laid by a given hen. Additionally, a weighting variable was calculated as follows:

$$w_i = (r_i)(n_i - r_i) \div (n_i - 1).$$

Transformed data were analyzed with a log odds model,

$$p(x) = 1 \div [1 + e^{-1(x)}],$$

where $0 \leq p \leq 1$ and $1(x) = \mu + \alpha_i + \beta_j + \gamma_k +$

$\delta_l + \varepsilon_{ijkl}$. The parameters α_i, β_j, γ_k, and δ_l represented male, female, male by female interaction, and replicate effects, respectively. The μ and ε were the overall mean and random error, respectively. Parameters were estimated with the General Linear Models procedure (SAS Institute, 1986).

Percentages derived from 7-day egg collection intervals were transformed to modified logits, $L*_i$, as follows:

$$L*_i = \log_e[(r_i - .5)(n_i - (r_i - .5))].$$

Likewise, modified weighting variables were calculated,

$$w*_i = (r_i)(n_i - (r_i - .5)) + (n_i - 1).$$

Transformed data were analyzed with the log odds model described above.

Duration of fertility was evaluated according to Kirby and Froman (1990). In brief, data from replicate 21-day egg collection intervals were pooled and plotted as a function of time. The parameters of

$$y(x) = \gamma + [1 + e^{\beta(\tau - x)}],$$

were estimated by iterative least squares (Kirby and Froman, 1990). Extra sums of squares F tests were made to determine whether estimates of τ, the time of half-maximal fertility, were estimates of a common parameter.

RESULTS

Analysis of data from the 7-day egg collection interval showed no difference ($P>.05$) in fertility between lines of males or lines of females (Table 1). Neither a male by female interaction nor a replicate effect was observed ($P>.05$). Analysis of data from the 21-day egg collection interval revealed a significant difference ($P<.05$) between LF and RB males as well as between LF and RB females. Neither a male by female interaction nor a replicate effect was observed ($P>.05$) when data from the 21-day egg collection interval were analyzed. Errors were independent and randomly distributed in both analyses.

Estimates of τ are shown in Table 2. When hens were inseminated with semen from RB males, the difference in τ between hen lines was 1.31 days ($P<.001$). Likewise, when hens were inseminated with semen from LF males,

TABLE 1. *Summary of fertility[1] following insemination of low-fertility (LF) or randombred (RB) Delaware hens with spermatozoa from LF or RB roosters*

			Egg collection interval			
			7 days		21 days	
Sire	Dam	Hens	Eggs	Fertility	Eggs	Fertility
		(n)	(n)	(%)	(n)	(%)
LF	LF	35	215	79.9 ± 3.2	566	35.9 ± 2.0
	RB	36	241	87.6 ± 2.9	615	44.4 ± 2.2
RB	LF	30	200	87.9 ± 3.3	499	47.7 ± 2.8
	RB	30	182	90.1 ± 2.7	466	52.4 ± 2.4
LF	Both	71	466	84.8 ± 2.2	1,181	40.4 ± 1.6[b]
RB	Both	60	382	89.0 ± 2.1	965	50.0 ± 1.9[a]
Both	LF	65	415	83.6 ± 2.3	1,065	41.5 ± 1.9[b]
Both	RB	66	423	88.7 ± 2.0	1,081	48.0 ± 1.7[a]

[a,b]Means ± SEM within sire and dam groups with no common superscripts were different (P<.05).

[1]Each percentage is a $\bar{x}$ ± SEM from three replicate trials.

the difference in τ between hen lines was 2.2 days (P<.0001). In either case, duration of fertility was greater for RB hens. Predicted fertility curves are shown in Figures 1 and 2.

DISCUSSION

When eggs are collected over an adequate interval following a single insemination, iterative least squares provides information that is easily interpreted. The parameters of interest are γ and τ. Parameter γ, a percentage, is an estimate of the initial level of fertility. Parameter τ is the time of half-maximal fertility in days. The other parameter estimated with this technique, i.e., β, has value only with respect to its sign. A negative β denotes a function that falls whereas a positive β denotes a function that rises. Thus, in the case of a fertility curve, β should always be negative.

In contrast to previous studies, which have shown that τ is affected by male genotype (Kirby *et al.*, 1989; Froman *et al.*, unpublished data), insemination site (Kirby *et al.*, 1989, 1990), and the state of the spermatozoon's glycocalyx (Froman and Engel, 1989), the present work demonstrates that τ also is affected by type of female (Table 2). It seems likely that this effect is related to spermatozoal sequestration within the hen's uterovaginal glands.

The difference in duration of fertility shown in Figures 1 and 2 cannot be attributed to different insemination doses. Furthermore, it is

unlikely that the RB hen's oviduct decreases the extent to which spermatozoa from LF males degenerate within the oviduct (Kirby *et al.*, 1989). Estimates of τ following intravaginal insemination of Leghorn hens with spermatozoa from LF males (Kirby *et al.*, 1989, 1990; Kirby and Froman, 1990; Froman and Engel, unpublished data) have averaged 9.45 days

FIGURE 1. Duration of fertility after intravaginal insemination of randombred Delaware (▲) and low-fertility Delaware hens (△) with extended semen from randombred Delaware roosters. Each hen was inseminated with 1×10^8 viable spermatozoa. Solid lines represent the functions $y(x) = 91.7 + [1 + e^{-.698(9.33 - x)}]$ for low-fertility hens and $y(x) = 86.8 + [1 + e^{-.621(10.64 - x)}]$ for randombred hens.

TABLE 2. *Estimates of τ^1, time of half-maximal fertility, and related statistics following a single insemination of randombred (RB) and low-fertility (LF) Delaware hens*

Semen source	Hens	τ	SSE[2]	CSSE[3]	F[4]	Probability $> F$
RB	RB	10.64	11,432.4	12,390.8	10.31	.001
RB	LF	9.33				
LF	RB	9.03	10,322.7	11,909.1	18.44	.0001
LF	LF	6.83				

[1]Estimated by iterative least squares from data obtained from a 21-day egg collection interval.

[2]Unconditional sum of squared residual errors (SSE) on $(n - p)$ degrees of freedom, where n denotes the number of observations and p denotes the number of parameters within the observational model, viz. γ, τ, and β.

[3]Conditional sum of squared residual errors (CSSE) on $(n - p + r)$ degrees of freedom, where r denotes the number of independent parametric statements implied by the condition. In each case above, the hypothetical condition was $\tau_{RB} = \tau_{LF}$, or $r = 1$.

[4]The F value was calculated as follows: $f_{r,(n-p)} = [SSH/r] + [SSE \div (n - p)]$, where SSH denotes the difference between CSSE and SSE.

with a coefficient of variation of 6.3% (n = 4). Therefore, the τ of 9.03 days for LF males bred to RB hens (Table 2) is indicative of subfertility, even though the estimate is greater (P<.0001) than that observed when LF males were bred to LF hens (Table 2). In view of the work of Van Krey et al. (1971), it is postulated that the difference in duration of fertility depicted in Figures 1 and 2 is attributable either to a diminution of the number of uterovaginal glands in LF hens or the extent to which these glands are filled with spermatozoa.

Data sets used to assess duration of fertility by iterative least squares are also suitable for the analysis of overall fertility with a log odds model, which compensates for lack of additivity (Kirby and Froman, 1990). In contrast, if a routine artificial insemination frequency is followed, overall fertility can be evaluated but τ cannot be estimated. Short-term egg collection intervals always yield greater percentages of fertilized eggs than long-term intervals (Table 1), and thus may seem to be preferable as a means of estimating reproductive potential. However, the present work demonstrates that this is not the case. Differences between males and differences between females were detected when data from 21-day egg collection intervals were analyzed. In contrast, no such differences were detected when data were restricted to eggs collected throughout the 1st wk following insemination, even though roosters in one line previously had been shown

to be subfertile (Froman and Bernier, 1987; Kirby et al., 1989, 1990).

This discrepancy cannot be attributed to different formulations for logits and weighting variables; for the calculation of these values must be varied empirically according to the distribution of data or errors (Anscombe, 1956; Govindarajulu, 1988). For example, when
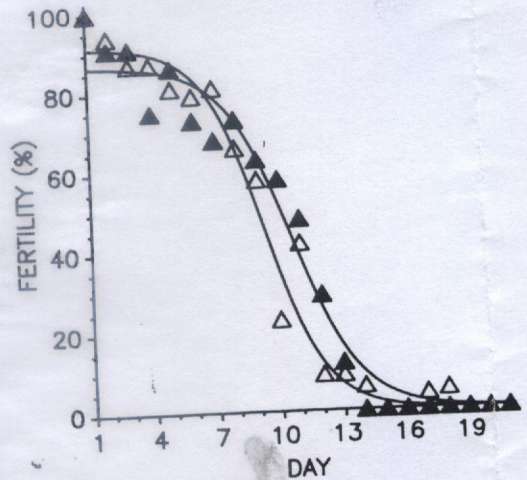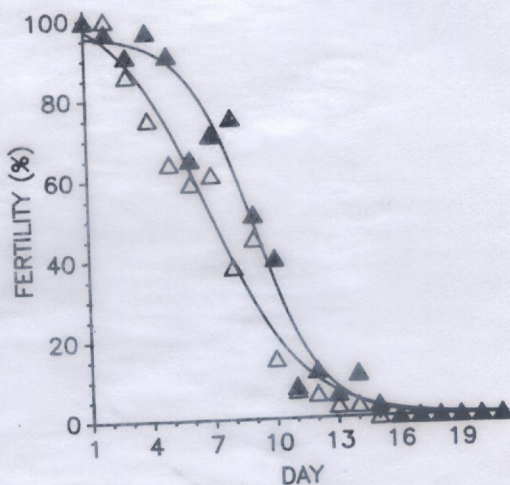

FIGURE 2. Duration of fertility after intravaginal insemination of randombred Delaware (▲) and low-fertility Delaware hens (△) with extended semen from low-fertility Delaware roosters. Each hen was inseminated with 1×10^8 viable spermatozoa. Solid lines represent the functions $y(x) = 105.0 + [1 + e^{-.438(6.83 - x)}]$ for low-fertility hens and $y(x) = 96.3 + [1 + e^{-.615(9.03 - x)}]$ for randombred hens.

percentages derived from 7-day egg collection intervals were transformed and weighting variables determined according to Kirby and Froman (1990), 67 hens had to be excluded from the data set. Each of these hens had laid an egg daily throughout the 7-day interval. When $n_i = r_i$, L_i and w_i became negative and zero, respectively, which precluded analysis. An additional complication arose in that the remaining data set was characterized by a skewed distribution of errors. In contrast, the modified transformation and weighting afforded the inclusion of all hens along with independent and randomly distributed errors.

As a corollary to the analysis of data from the 7-day egg collection interval, this same data set was arc sine transformed. As expected, residual errors were characterized by a heteroscedastic distribution as described for fertility data by Chaudhary *et al.* (1984). In summary, if it had not been known *a priori* that one male line was subfertile and had data collection been limited to a 7-day interval, the reproductive status of the LF males would have appeared to be equivalent to that of RB males. Also, the difference between females would have been missed.

Therefore, the present work demonstrates that the interpretation of fertility data may be complicated by use of an inadequate sampling interval. A complete and robust analysis is afforded by 1) an adequate sampling interval; 2) data analysis by iterative least squares; and 3) data analysis with the log odds model. These methods may be used to assess how fertility is affected by either the male or female. Finally, time of half-maximal fertility provides an additional selection criterion for improving duration of fertility.

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PROCESSING AND PRODUCTS

Lipid Composition of Hexane and Supercritical Carbon Dioxide Reduced Cholesterol Dried Egg Yolk[1,2,3]

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ABSTRACT   Cholesterol was extracted from commercially spray-dried egg yolk using hexane at atmospheric pressure or supercritical carbon dioxide ($SCCO_2$) (45 C, 238 atm; 45 C, 306 atm; 55 C, 374 atm). The effects of the different extraction methods on the lipid composition of the cholesterol-reduced yolks were evaluated. Polar lipids were separated into seven fractions using HPLC. Fractions 4, phosphatidylethanolamine (PE), and 5, phosphatidylcholine plus lysophosphatidylethanolamine (PC+LPE), accounted for almost 85% of the phospholipid (PL). As $SCCO_2$ temperature and pressure increased, total lipid remaining decreased from 64.9 to 35.1%, but the PL content (15%) remained unaffected. The proportions of PE (12%) and PC+LPE (72%) of the PL fraction were constant. There was little effect on the fatty acid composition of the phospholipid fractions due to the $SCCO_2$ treatments. However, the unsaturated fatty acids in the neutral lipids (NL) appeared to be effected by $SCCO_2$ treatments. Hexane extracted a larger amount of total lipid (66%) and the NL fraction was decreased more than the PL fraction. Supercritical carbon dioxide was more efficient in removing cholesterol without major effects on the PL.

(*Key words:* cholesterol, egg yolk, lipids, supercritical carbon dioxide, extraction methods)

INTRODUCTION

Consumption of eggs and egg products has been affected by concern about dietary cholesterol. Many consumers are no longer including eggs in their diet because so much emphasis by health groups has been placed on the high cholesterol content of eggs. Many low-cholesterol egg substitutes have been produced. These products contain egg white, milk solids, vegetable oil, and little or no egg yolk. If processors continue this trend towards making egg products containing only egg white, a surplus of egg yolk may result. Consumption of such products will not increase the consumption of whole eggs. Development of products containing egg yolk and white would be a more favorable way to increase the consumption of eggs. Extraction of cholesterol from egg yolks and the subsequent use of the residual yolk proteins and lipids is an alternative to development of low-cholesterol egg products.

Several researchers have extracted the cholesterol from egg yolk with organic solvents (Melnick, 1971; Larsen and Froning, 1981; Tokarska and Clandinin, 1985; Warren *et al.*, 1988). Tokarska and Clandinin (1985) extracted the lipid and cholesterol from egg yolk for use in liquid formulas. A yield of 93% of the total egg yolk lipid was accomplished by using egg yolk:ethanol:hexane:water in a 1:2:1:1 (wt/vol/vol/vol) ratio and centrifuging. After refining, the final product contained 7 mg cholesterol/g of oil. Tokarska and Clandinin (1985) did not discuss the residual yolk protein portion, however.

Melnick (1971) extracted the lipid and cholesterol from egg yolk solids by slurrying