

ASSESSING THE EFFECT OF MATING RATIO ON BROILER BREEDER PERFORMANCE BY QUANTIFYING SPERM:EGG INTERACTION

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Primary Audience: Breeder Managers, Researchers

SUMMARY

Trials to compare mating ratios are important for optimizing the breeding efficiency of broiler breeder flocks. However, the parameter that is normally used for such comparisons—'fertility' of all eggs or a sample of eggs from the flock—is a rather coarse tool for discriminating the interplay of mate choice and mating frequency, which results from different male:female ratios. In this pilot study, we have shown that the number of holes hydrolyzed by spermatozoa in the inner perivitelline layer over the germinal disc in samples of 40 eggs is better than measures of flock or sample fertility for discriminating between pairs of flocks with different proportions of males. For example, the median number of holes made by spermatozoa in the perivitelline layer of samples of eggs can be doubled in flocks with 9.5 versus 9.0 males per 100 females, which shows that flock fertility increases less than 1%. The perivitelline hole assay also provides an indication of the distribution of spermatozoa among hens, which is the fundamental parameter for assessing the dynamics of mating efficiency under different male:female ratios. The number of spermatozoa transferred to, and their distribution among, hens then defines flock 'fertility.'

Key words: Broiler breeder, fertility, mating ratios, sperm:egg interaction

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DESCRIPTION OF PROBLEM

In commercial broiler breeder flocks, the proportion of males to females is an important parameter for maximizing fertility. Breeders generally recommend around 8 to 9 males per 100 females at 20 to 30 weeks of age with a reduction to 6 to 7 males by the end of the laying period [1, 2, 3]. At greater than 10 males per 100 females, fertility may be adversely affected by excessive male aggression and com-

petition for mating and territory [3, 4, 5]. Although ratios as low as 7 males per 100 females can give adequate fertility in older flocks [1, 2, 3], there is a danger that, in some circumstances, there may be insufficient males to impregnate an acceptable number of females.

A major problem for determining the optimal mating ratio for any flock is that fertility is a crude measure and does not allow further analysis of mating dynamics, which result from different male:female ratios. An alternative,

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more sensitive and informative parameter of mating efficiency is the assay of sperm transfer into eggs [6, 7, 8]. This parameter reflects the number of spermatozoa transferred from the male to the female and can present, for each egg, a scale from 1 to over 1,000 [9], which is a considerable improvement on the binary fertilized/not-fertilized assessment. Therefore, we have applied a test of sperm:egg interaction to follow three cohorts of commercial broiler flocks (A, B, C), each held in two houses (sites) at different male:female ratios from 18 weeks of age.

MATERIALS AND METHODS

Three cohorts of broiler breeders, designated as A, B, and C, were studied. These were Ross 308 females and Ross males from unrelated hybrid lines. Until 18 weeks, males and females were reared separately according to the international version of the Ross Manual [2] in 8 hr of darkness per 24-hr day. At 18 weeks of age, each cohort of females was divided and housed with males at different numbers of males per 100 females, which is noted in the subscripts of the flocks: A_{8.4}, A_{8.9}, B_{9.0}, B_{9.5}, C_{9.0}, and C_{9.5}. At this time, the photoperiod was adjusted to 16 hr darkness per 24 hr of light, and birds were fed separately the same commercial breeders ration. Samples of 40 eggs from each flock at 30, 40, and 50 weeks were sent by

carrier to the University of Abertay, where they were stored at 5 °C for up to 2 weeks before analysis.

Eggs were brought to room temperature and cracked open. Each egg was assessed for fertility by the Kosin test: a macroscopic observation of the germinal disc/blastodisc [10]. Then, a piece (approximately 1 cm square) of perivitelline layer from around the germinal disc was removed, washed free of yolk, and spread on a microscope slide; a coverslip was placed on top [6]. The number of holes hydrolyzed by spermatozoa in the inner perivitelline layer (IPVL) in the region over the germinal disc (GD-IPVL holes) [9] was counted under dark-ground optics.

In a few cases in which the Kosin test did not provide a definitive demonstration of fertility, eggs were deemed to be fertile when they had more than six GD-IPVL holes and were deemed infertile when they showed no GD-IPVL holes [9]. In the rare cases when such eggs were found to have one to five GD-IPVL holes, the result of the original Kosin test was used. Eggs shown to be fertile using this method were combined with those determined to be fertile using the Kosin method to define 'sample fertility.' The company provided flock fertility, assessed by candling and breakout of 'clears.'

The sign test was used to compare the medians from the same flocks with different male:female ratios at the 5% significance level.

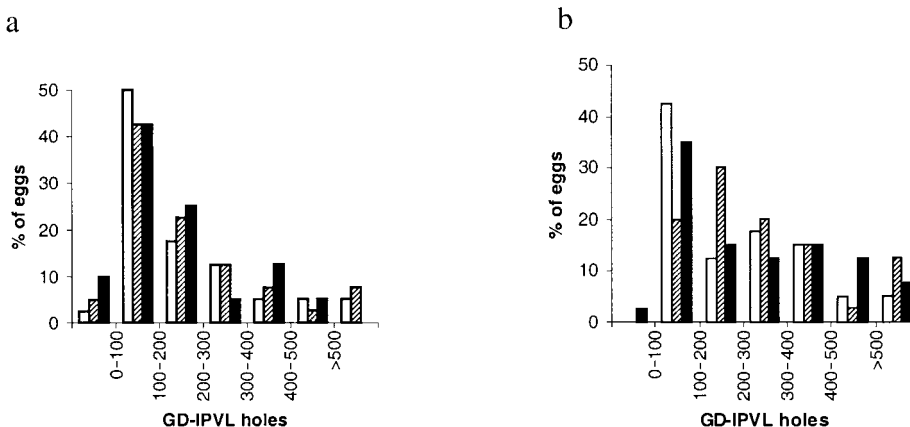


FIGURE 1. Frequency distribution of holes in the inner perivitelline layer (IPVL) over the germinal disc (GD-IPVL holes) in samples of eggs from Cohort C. Graphs (a) and (b) show GD-IPVL-holes from flocks with 9 and 9.5 males per 100 females, respectively. Samples were taken at 30 weeks (open bars), 40 weeks (hatched bars), and 50 weeks (black bars). See Table 1 for flock details.

TABLE 1. Fertility and median GD-IPVL holes (holes hydrolyzed by spermatozoa in the inner perivitelline layer in the region over the germinal disc) from all flocks at all ages

COHORT	FLOCK AGE	MALES PER 100 FEMALES	FLOCK FERTILITY		SAMPLE FERTILITY		MEDIAN GD-IPVL HOLES
			(%)				
A	30	8.4	93.0	97.5	112		
		8.9	94.2	95.0	128		
	40	8.4	92.4	97.5	108		
		8.9	94.3	97.5	138		
	50	8.4	86.7	83.3	90		
		8.9	87.0	90.0	118		
B	30	9.0	92.6	90.0	74		
		9.5	92.5	97.5	100		
	40	9.0	93.6	93.3	72		
		9.5	94.0	96.6	154		
	50	9.0	88.5	90.0	76		
		9.5	93.7	95.0	108		
C	30	9.0	96.0	97.5	76		
		9.5	94.7	100	138		
	40	9.0	92.3	95.0	130		
		9.5	93.0	100	206		
	50	9.0	85.7	87.5	92		
		9.5	87.6	95.0	188		

RESULTS AND DISCUSSION

The distribution of GD-IPVL holes in samples of 40 eggs from all flocks were found, similar to findings from previous work [9], to be positively skewed; the degree of skewedness in the present samples was greater in low mating ratio groups (see Figure 1 for an example). Therefore, the median was used as the appropriate summary statistic for these samples.

In all flocks, at each age, the median number of GD-IPVL holes was greater in the higher mating ratio groups, although this was not always true for flock or sample fertility (see Table 1). The one-tailed sign test showed that differences between the high and low male:female ratio flocks for the median GD-IPVL holes and sample fertility were significant ($p = 0.002$ and $p = 0.0352$, respectively); those for flock fertility were not significant ($p = 0.0898$). Averaged over all ages, the increase in the median GD-IPVL holes was 24.3, 63.7, and 81.3% for Cohorts A, B, and C, respectively; the increase in sample fertility was 1.8, 5.8, and 5.4%, and the average increase in flock fertility was 1.2, 2.1, and 0.5%. Thus, compared with median GD-IPVL holes, flock fertility was a more limited

and less sensitive measure of the differences between low and high mating ratio groups. An interaction plot of the median GD-IPVL holes pooled from Cohort C (see Figure 2) demonstrates the greater differences between mating ratios at 50 weeks of age compared with those at 30 and 40 weeks of age.

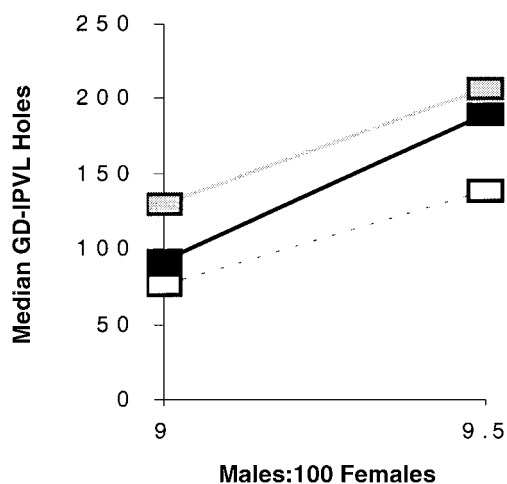


FIGURE 2. Age effects on median GD-IPVL holes for different mating ratios in cohort C. Samples were taken at 30 weeks (open symbols), 40 weeks (gray symbols), and 50 weeks (black symbols). See text for details.

The regression line for flock fertility (y) on sample fertility (x) was $y = 0.53x + 41$ ($r = 0.77$; $p < 0.05$). Flock fertility was consistently lower than sample fertility by about four units at higher sample values; whereas, at lower values, this difference was zero or negative. Although this may be a sampling effect, it might also reflect a proportion of incubated eggs that are erroneously assessed as 'infertile' because incubated 'clears' might include embryos that died at an early stage. However, why the difference between flock and sample fertility is not found at lower values remains unknown and may suggest an overestimation of flock fertility.

The relationship between flock fertility and sample median GD-IPVL holes is variable but in general agreement with that found previously [8]. Mismatches between median GD-IPVL

holes and fertility may be the result of differences in the distribution of spermatozoa among eggs rather than in the total numbers of spermatozoa found in the eggs. This, in turn, reflects an uneven distribution of spermatozoa among hens, which is a likely result of different mating frequencies. We have previously found that the main source of infertile eggs in floor-mated broiler breeder hens is a distinct subpopulation of hens that do not appear to be mating [8]. This subpopulation can be observed to have zero sperm in the distribution of GD-IPVL holes as shown in Figure 1.

These distributions of spermatozoa among eggs or hens are important considerations when designing systems aimed at determining the proportion of males for optimizing broiler breeder fertility and, at present, are best determined through assay of sperm in eggs.

CONCLUSIONS AND APPLICATIONS

1. This pilot study indicates that a sperm:egg interaction assay in samples of eggs from floor-mated broiler breeder flocks is more discriminatory than flock fertility for defining differences between pairs of flocks with different mating ratios.
 2. This assay also allows analysis of the distribution of sperm among eggs, reflecting differences among hens, and its relationship to mating ratios and fertility.
 3. The assay may find application 'in the field' because it requires only limited bench space and an inexpensive microscope.
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