Does Evaluation of One or Two Ejaculates of Rooster Semen Provide a Valid Basis for Culling Inferior Males?

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Primary Audience: Flock Supervisors, Reproductive Managers, Laboratory Personnel

SUMMARY

Despite the practice of spiking flocks with younger males, reproductive problems increasingly impact the capability of flock managers to produce needed chicks and increase the cost of chick production. The problem also impacts breeders and producers using artificial insemination (AI). Recognizing that the problem is partially caused by poor semen quality, flock managers might consider evaluation of young males and culling those whose semen is of low quality. This logically leads to two questions. What should be evaluated and how many ejaculates should be evaluated before deciding to cull a rooster or tom? Important semen attributes include volume and number of sperm per milliliter (concentration) plus attributes of sperm function such as motion, exclusion of vital dyes, capability to bind to the oviduct epithelium, or capability to bind to the perivitelline membrane of an ovum.

Herein, we focus on general aspects of gathering valid information on semen rather than what to measure, although roosters producing few sperm should be culled. Such a focus is to provide flock supervisors a rational basis to make decisions based on limited data.

To estimate predictive value, we used available data for three successive ejaculates from each of 114 broiler roosters in a pedigree flock. Data collected were number of sperm per milliliter of semen and capability of sperm to bind in an in vitro sperm-binding assay. The average for all three ejaculates was used to identify those roosters in the lowest 20% for each trait.

For both tests, the odds of making the correct culling decision based on one ejaculate was less than 2:1 (erroneous conclusion was made >35% of time). For the binding assay, but not sperm concentration, evaluation of two successive ejaculates increased the odds of a correct decision to greater than 3:1. When assessing these traits, we recommend evaluation of three successive ejaculates to enable a flock supervisor to usually, with reasonable reliability, make correct decisions to cull or not cull a rooster.

Key words: Culling roosters or toms, decision-making, semen evaluation

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DESCRIPTION OF PROBLEM

Reproductive performance lower than desired is not a new problem, and there is a perception in the industry that the problem is increasing. A reproductive problem is recognized by a relatively low percentage of eggs set providing a chick or poult. The cause usually is not obvious, but it could be the males (many with poor semen quality; roosters mating with few hens), females (many ovulating ova with a low capability to be fertilized; low rate of lay), or management practices.

There is no practical approach to detect and cull subfertile hens from large flocks. It is possible to cull males producing inferior semen, although traditionally this practice is not common with chickens because it requires collection of semen. With turkeys, semen is collected from all toms, but the semen is not routinely evaluated for quality. Primary biological causes underlying the problem of suboptimal reproductive performance are known. Ignoring the problem will not solve it.

Flock managers are increasingly receptive to consider culling of potentially subfertile males, because biotechnology is providing new or improved methods for evaluation of semen quality. The need to collect semen cannot be avoided. A meaningful use of outcome data from a validated laboratory diagnostic test for subfertility (i.e., proven to be highly predictive) would allow a flock supervisor to cull the roosters or toms that are least fertile (possibly the lowest 15 to 20%) before placing the remaining males with hens or using their semen in pools used for AI.

It is beyond the scope of this paper to review semen analysis, as there are several excellent handbooks and texts [1, 2] on this subject plus recent research reports [e.g., 3, 4, 5]. In summary, the important semen attributes are lack of obvious contamination, volume, number of sperm per milliliter (concentration), and total number of sperm per ejaculate. Important attributes of sperm function include motion (e.g., percentage of motile cells, wave action, or mobility), percentage excluding vital dye, capability to bind to the oviduct epithelium, or capability to bind to the perivitelline membrane of an ovum.

With poultry, as with livestock and humans, judgment of semen quality is based on the total information available for a given sample. For poultry, objective measures of sperm motion and concentration now are available [1, 2, 3, 5, 6, 7] and should replace subjective evaluation of sperm motion or color. Augmentation with a measure of sperm-egg binding [4, 8] would increase the information base for quality of any given sample.

Semen quality cannot be based on a single type of evaluation, although a given spermatozoon might fail to fertilize an ovum due to failure of one functional attribute. Other sperm might fail for a different reason [9, 10]. Hence, conclusions based on evaluation of only one functional attribute cannot identify males producing a majority of sperm functionally incapable of fertilizing an ovum.

Several tests should be used, and those selected should provide objective outcomes that are only weakly correlated (e.g., $r < 0.35$). If the outcomes from tests are highly correlated, they are essentially measuring the same features of sperm. Use of several laboratory assays, each evaluating a different functional attribute of sperm, and culling males predicted to be subfertile on the basis of outcome in any individual test (or an index based on several outcomes) can increase fertility. This increased fertility will have a biological impact as well as an economic one.

For practical reasons, evaluation of a minimal number of ejaculates consistent with making the right decisions is essential. For >30 yr it has been known [11, 12, 13] that quality and especially quantity of sperm in ejaculates from an individual differ substantially on different days. Nevertheless, evaluation of only one ejaculate might appeal to a cost-conscious flock manager.

As reproductive biologists concerned about correct use of diagnostic assays, we considered evaluation of at least two, and preferably three, ejaculates as the minimum information on which to decide that a 25-to-30-wk-old rooster or tom was unfit for use in pen mating or unfit as a semen donor for AI. Hence, we tested the notion that evaluation of only one ejaculate, or two successive ejaculates, of rooster semen could

provide a basis for culling, equally as strong as a decision based on evaluation of three ejaculates collected within 5 d.

Our data set contained evaluations for one quantitative characteristic and one qualitative characteristic (see below), and modeling outcomes to test the above theory were illustrative and instructive. The reader should decide whether the conclusions are more generally applicable (other lines of birds, different personnel, different instruments, and different semen characteristics or diagnostic assays).

A procedure to evaluate sperm function would have little appeal to a flock supervisor, unless that assay has been validated and shown to be predictive of fertility over the following months. Calculation of a statistically significant correlation coefficient using data pairs for outcome in a laboratory test and estimated fertility for each member of a group of males does not validate a diagnostic assay [9, 10]. Correlation coefficients are used to evaluate past results within a population but cannot predict what any given individual will do in the future. Concepts of prediction, which evolved over 25 yr ago in respect to sperm quality and fertility, have not been used by poultry or animal scientists or by most veterinarians. However, these approaches are simple to understand [10, 14], and conclusions are expressed as probability or odds of reaching the right conclusion (e.g., culling of all males likely to have fertility lower than a selected value or retaining only males with a fertility greater than the selected value). Herein we include a contrast between the correlative and predictive approaches.

MATERIALS AND METHODS

Semen was collected [15] from roosters of a pedigreed flock from a commercial broiler breeder. Semen was collected twice weekly from all males for at least 2 wk before initiation of this study. For the study, each ejaculate was mixed by aspiration up and down a transfer pipette. Then subsamples of the semen were used to perform two quantitative tests of semen quality. These were concentration of sperm in the semen and capability of sperm to bind in vitro to a substrate containing an extract of chicken-egg perivitelline membranes.

Sperm concentration was determined [16] on the basis of light transmission using a densimiter. The digital display of billions of sperm per milliliter was recorded along with the leg-band number of the male. Semen volume was not determined.

Capability of sperm to bind to an egg membrane was evaluated [4, 17] with a relatively new diagnostic test, the DudFinder sperm-binding assay (SBA). The SBA is a commercial version of an assay to detect males whose semen contained a high proportion of sperm deficient in capability to bind to the perivitelline membrane of an ovum, the first step in the fertilization process. This defect is heritable [19]. Obviously, failure of the majority of sperm to bind to the perivitelline membrane precludes fertilization.

For each of 114 roosters providing all three ejaculates, data for sperm concentration and percentage of sperm bound were averaged across the three ejaculates. Males whose average values placed them in the lowest 20% of the population (23 roosters for each attribute) were identified as to be culled and were termed poor. The remaining 91 roosters were termed good. The CV for the three values for each rooster also was calculated.

We then determined the number of the 23 roosters designated for culling that had been in the lowest 20% of the first replicate of 114 ejaculates (i.e., lowest 23 values) and similarly for the ejaculates of the second and third replicates. Assuming that the result (poor or good; cull as potentially subfertile or retain as likely fertile) in the ranking based on the average for the three ejaculates was the correct result [20], we calculated the proportions of correct and incorrect conclusions that would have been made on the basis of any single ejaculate (i.e., first, second, or third) in the series. The true-positive, true-negative, false-positive, and false-negative rates [10, 14, 22] for prediction of poor roosters were calculated on the basis of any one ejaculate. We also calculated the odds of making a correct conclusion, with respect to the decision to cull (e.g., a positive test outcome), based on evaluation of a successive pair of ejaculates (i.e., average of Ejaculates 1 and 2 or 2 and 3).

RESULTS AND DISCUSSION

Quality of semen differed among the 114 roosters, showing that there was potential to cull inferior males. There was greater than fourfold

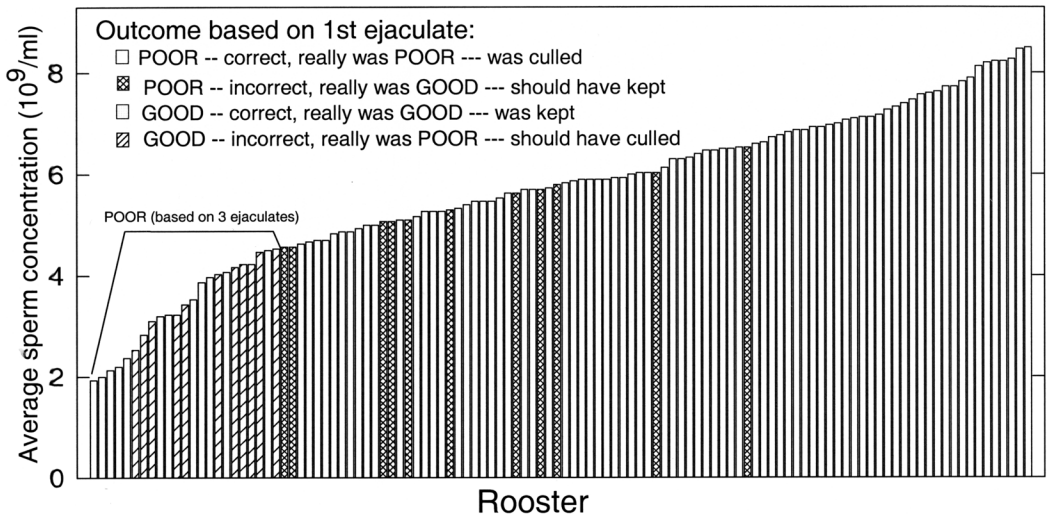

FIGURE 1. Average concentration of sperm in semen representing three ejaculates from each of 114 roosters. The bars designate correctness of a culling decision if it had been made on the basis of the first ejaculate [14, 20]. A true-positive outcome would be correct designation as poor (20% of population; left-most 23 males) and a true-negative outcome would be correct designation as good (right-most 91 males).

range in sperm concentration (Figure 1) and >11-fold range in percentage of sperm bound (Figure 2). For sperm concentration, average values ranged from 1.93 to 8.50 $\times$ 10^9 sperm/mL, and the CV for the three ejaculates contributing to each mean ranged from 3 to 116%. For sperm binding, average values ranged from 1.0 to 11.2% sperm bound, and the CV ranged from 3 to 129%. Values for these two characteristics were independent from each other, as the correlation between mean sperm concentration and mean percentage of sperm bound was 0.15 ($P = 0.12$).

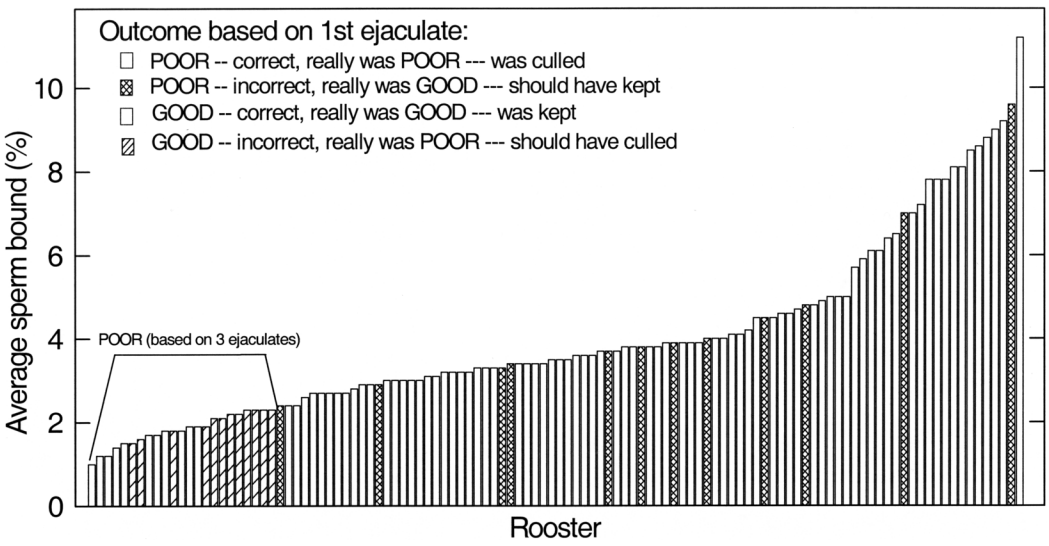

FIGURE 2. Binding capability of sperm in semen representing three ejaculates from each of 114 roosters. The bars designate correctness of a culling decision if it had been made on the basis of the first ejaculate [14, 20]. A true-positive outcome would be correct designation as poor (20% of population; left-most 23 males), and a true-negative outcome would be correct designation as good (right-most 91 males).

TABLE 1. Probability of correct decisions to cull or retain a rooster that was placed in the lowest 20% of the population on the basis of sperm concentration or sperm binding for only one ejaculate or on the basis of the average for two successive ejaculates [14, 20][A]

	Conclusion based on any one ejaculate[B]		Conclusion based on two successive ejaculates[C]	
	Sperm concentration	Sperm binding	Sperm concentration	Sperm binding
True-positive rate (correct cull of male)	0.65	0.58	0.50	0.76
True-negative rate (correct retention of male)	0.91	0.89	0.87	0.94
False-positive rate (incorrect cull of male)	0.09	0.11	0.13	0.06
False-negative rate (incorrect retention of male)	0.35	0.42	0.50	0.24

[A]"True" ranking was assumed to be that based on the average value for three ejaculates per rooster.
[B]Calculated by totaling outcomes for Ejaculate 1, 2, or 3.
[C]Calculated by totaling outcomes for average of Ejaculates 1 and 2 or average of Ejaculates 2 and 3.

The correlations between the values for the first ejaculate and the average for all three ejaculates for a rooster (based on 114 roosters) were 0.73 for sperm concentration and 0.59 for sperm binding. These correlations ($P < 0.001$) might be interpreted as evidence that evaluation of one ejaculate was sufficient. However, this conclusion would have been incorrect. For each attribute, the correlation shows the overall trend but not the correctness of the conclusion for each of the 23 poor roosters or 91 good roosters.

The lowest 20% of the average values for sperm concentration were those of $\leq 4.50 \times 10^9$ sperm/mL. Of these 23 poor roosters, 12 would have been detected on the basis of the first ejaculate, but 11 roosters would have been incorrectly designated for culling (Figure 1). Among the 91 good roosters that would have been designated for retention on the basis of the first ejaculate, 11 actually should have been culled.

The lowest 20% of the average values for sperm binding were those of ≤2.3% sperm bound. Of these 23 poor roosters, 11 would have been detected on the basis of the first ejaculate, but 12 roosters would have been incorrectly designated for culling (Figure 2). Among the 91 non-poor roosters that would have been designated for retention on the basis of the first ejaculate, 12 actually should have been culled.

Based on evaluation of a single ejaculate, the true-positive rates (probability of correctly culling a poor rooster) were 0.65 for sperm concentration and 0.58 for sperm binding (Table 1). The true-negative rates (probability of correctly retaining a good rooster) were 0.91 for sperm concentration and 0.89 for sperm binding, but the false-negative rates (probability of incorrectly retaining a poor rooster) were 0.35 and 0.42. The probabilities of culling the right roosters on the basis of one ejaculate were unacceptably low. Acceptable values for true-positive and true-negative rates would be ≥0.85 and often ≥0.90, although acceptability depends on the use of the outcome from a diagnostic test.

When two successive ejaculates were used to make a decision for percentage of sperm bound, the true-positive rate was 0.76 rather than only 0.58 (Table 1), and the true-negative rate remained high. For sperm concentration, however, evaluation of two successive ejaculates rather than only one ejaculate did not improve predictive power. These divergent results likely reflect the nature of the two measurements. Sperm concentration is a characteristic of semen and, in part, reflects skill of the semen collector and the amount of transparent fluid obtained in addition to number of cells in the sperm-rich material. This trait likely is influenced substantially by factors that are not biological. Sperm binding, on the other hand, is a functional characteristic of individual sperm making up the population within an ejaculate. It is unlikely that, in healthy birds, this trait would differ severalfold from one ejaculate to the next. Indeed, measurements of sperm binding are repeatable over time, and the trait is heritable [19].

We rejected the notion that evaluation of one ejaculate was sufficient, because the odds of making the right decision were <2:1 that a given poor rooster would appear in the bottom 20% of the population due to any one ejaculate and, hence, be selected for culling. This finding

was true for decisions based on sperm concentration or the sperm-binding assay.

CONCLUSIONS AND APPLICATIONS

1. Given the wide range in quality of semen produced by a typical population of young roosters, it is logical that fertility of males used for pen mating could be improved by culling the males with the lowest fertility.
2. Flock managers might consider culling up to 20% of all young males, as the value of a higher hatch per egg set could exceed the value of the males culled.
3. If roosters are to be culled on the basis of sperm concentration or sperm binding, the decision never should be based on a single ejaculate. This conclusion should apply regardless of the instrument used to measure sperm concentration. Given the cited literature on variation of semen or sperm characteristics among ejaculates from individual males, this conclusion likely would apply to any measure of semen quality.

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17. The DudFinder® Sperm-Binding Assay (SBA; BioPore Inc, State College, PA) is a commercial version of Assay 3 detailed by Barbato et al. [4]. It was conducted as described by those authors. In brief, an aliquot of each ejaculate was diluted to 20×10^6 sperm/mL with MnA extender [18], and 100-μL aliquots were dispensed into a series of microwells in an SBA plate. After 60 min of incubation at 32°C, unbound cells were washed away, and the plates were dried. Number of bound sperm was determined later, as in Barbato et al. [4].

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true-positive rate = number poor with positive test based on one ejaculate/total poor

true-negative rate = number good with negative test based on one ejaculate/total good

false-positive rate = number good with positive test based on one ejaculate/total good

false-negative rate = number poor with negative test based on one ejaculate/total poor Although we did report values, one also could calculate

predictive value positive = number poor with positive test based on one ejaculate/total with positive test

and

predictive value negative = number good with negative test based on one ejaculate/total with negative test.

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