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Use of Nonsettable Eggs to Evaluate Turkey Hen Fertility

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Primary Audience: Hatchery and Farm Personnel, Poultry Scientists

SUMMARY

The use of nonsettable eggs (checked, cracked) to perform fresh egg breakouts to estimate true fertility and to assess the rate of sperm penetration of the perivitelline layer overlying the germinal disc was evaluated. Germinal discs and the perivitelline layer overlying the germinal disc were accessible for assessments. The stage of blastodermal development positively correlated ($r = 0.65$, $P < 0.0001$) with eggshell thickness. It was also determined that the perivitelline layer of nonsettable eggs could be isolated and stained to determine the presence or absence of sperm holes. True fertility of nonsettable eggs (checked and cracked only) and settable eggs was 90 and 95%, respectively. It was concluded that checked and cracked eggs could be used to estimate the true fertility of a flock.

Key words: egg fertility, embryo, turkey

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DESCRIPTION OF PROBLEM

The need to differentiate fertilized from unfertilized germinal discs is of paramount importance when determining the basis of a poor hatch. For effective management of hatching eggs, quality control measures such as fresh egg breakouts [1] should be used routinely to get a snapshot of true fertility. If true fertility is poor in freshly laid eggs, management can take immediate corrective action. This action includes inseminating increased sperm numbers or "doubling-up" on the inseminations (two inseminations in one 5-d period).

Notwithstanding that hatching turkey eggs cost about $0.55 per egg to produce [2] nonsettable eggs are generally discarded or rendered. In an effort to reduce the cost of egg production and increase production revenue, we evaluated the use cull eggs for determining true fertility. As a secondary objective, we evaluated the stage of development of the blastoderm in soft shell and membranous eggs. Gupta and Bakst [3] showed a positive correlation between the stage of embryo development and eggshell thickness while the egg mass was in the uterus. By staging the embryo of soft shell and membranous eggs we will be able to determine if these eggs are expelled prematurely from the oviduct or reside in the uterus for the duration of the ovulatory cycle.

MATERIALS AND METHODS

Eggs from Large White turkey hens, which were maintained under standard husbandry conditions at the Beltsville Area poultry farm, were

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brought to the hatchery three times daily. Hens had been inseminated weekly with about 250 million sperm. All soft-shelled, shell-less, and cracked eggs were stored in flats in a sealed plastic bag in an 18°C cold room for no longer than 6 d. Breakouts were performed (see [1]), and the germinal disc was examined by stereomicroscopy. Differentiation of fertilized and unfertilized germinal discs was based on criteria adopted in the work by Bakst et al. [4]. The perivitelline layer (PL) overlying the germinal disc was isolated and sites of sperm penetration (PL-sperm hole) determined using the method of Howarth and Donoghue [5].

Shell thickness of the eggs (n = 53) was based on the average of three caliper measurements of the shell membrane plus shell (if present) at equidistant points around the equatorial region of the egg. Statistical analysis [6] determined the degree of correlation (Pearson's correlation analysis) between eggshell thickness and the stage of embryonic development.

The true fertility of settable (n = 132) and nonsettable (n = 287) eggs from one flock on a commercial farm [7] was also determined. Large White hens, which were 17 wk into egg production, were inseminated weekly with about 350 million sperm. The semen was diluted with Beltsville Poultry Semen Extender and held less than 5 h. Cull eggs, which were limited to cracked and checked eggs, were stored at 55°F (18°C) for no longer than 6 d in plastic bags and broken out as described above.

Percentage fertility was determined for the following groups: control unincubated (n = 71), control incubated (n = 61), cull-thin-shelled unincubated (n = 108), cull-cracked unincubated (n = 158), and cull-cracked incubated (n = 21). Each egg was considered to be an independent observation, therefore individual eggs were considered to be the sampling unit upon which sample size was determined. Standard error was estimated for each treatment group using the equation SE = square root [(p)(q)/n], where p = percentage fertile eggs, q = percentage infertile eggs, and n = number of eggs used to estimate fertility for a given treatment. Chi-squared analyses (PROC FREQ, SAS version 8.2; [6]) were conducted as pairwise comparisons to detect any differences between the fertility estimates. Significance was accepted at $P < 0.05$.

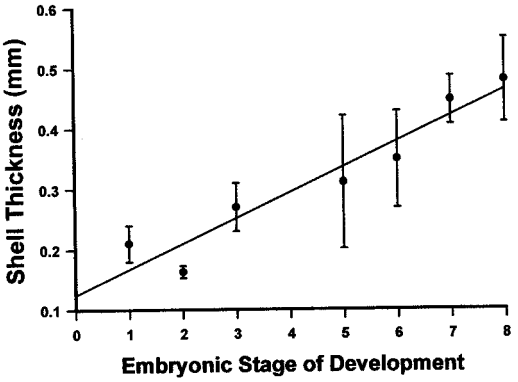

FIGURE 1. There is a positive correlation ($P < 0.01$) between the embryonic stage of development described by Gupta and Bakst [3] and eggshell thickness in nonsettable, fresh-laid eggs.

RESULTS AND DISCUSSION

Identification of the stage of embryo development was readily performed with the culled eggs if the eggs were not dehydrated. If dehydrated, the germinal disc could not be easily isolated, and fertility determination was hampered. There was a strong positive correlation ($r = 0.65$, $P < 0.0001$) between shell membrane/eggshell thickness and the stage of development of the blastoderm when culled eggs were broken-out (Figure 1). Isolation and staining of the PL and the subsequent visualization of the PL-sperm holes from all culled eggs were identical to that of settable eggs.

The Wampler eggs' true fertility for each egg category is shown is Table 1. Cull-cracked unincubated and cull-cracked incubated eggs had true fertilities of 88.6 and 90.5%, respectively. Cull-thin-shelled eggs had a true fertility of 88.9%. Chi-squared analyses conducted as pairwise comparisons of each cull-egg true fertility combination revealed no statistical differences (Table 1). The implication here is that an erratic ovulatory cycle, as manifested by ovipositing a thin-shelled egg, had no effect on fertilization. The settable control eggs that were either unincubated or incubated had true fertilities of 91.5 and 98.4%, respectively. Although chi-squared analysis showed no statistical difference between these values (Table 1) the *P*-level approached significance ($P = 0.082$). The nearly 7% difference between the settable incubated and unincubated eggs could be due of the distri-

TABLE 1. True fertility of various categories of turkey eggs

Item	Cull-cracked unincubated	Cull-cracked incubated	Control unincubated	Control incubated	Cull-thin shelled unincubated
Number fertile	140	19	65	60	96
Number infertile	18	2	6	1	12
Percentage fertility	88.6	90.5	91.5	98.4	88.9
SE	2.5	6.4	3.3	1.6	3.0

Summary of *P*-values	df	Value	*P*
Control incubated vs. control unincubated	1	3.03	0.08
Control incubated vs. cull cracked incubated	1	2.75	0.10
Control incubated vs. cull thin	1	4.92	0.03
Control incubated vs. cull cracked unincubated	1	5.28	0.02
Control unincubated vs. cull thin	1	0.33	0.56
Control unincubated vs. cull cracked unincubated	1	0.45	0.50
Control unincubated vs. cull cracked incubated	1	0.02	0.88
Cull cracked unincubated vs. cull thin	1	0.01	0.94
Cull cracked unincubated vs. cull cracked incubated	1	0.06	0.80
Cull cracked incubated vs. cull thin	1	0.05	0.83

bution of the eggs, or it could reflect errors in the visual differentiation between fertilized and unfertilized germinal discs. However, we believe the latter is unlikely because if there was doubt regarding the status of the germinal disc, we would extract material from the germinal disc, mix it with a fluorescent nuclear stain, and observe the presence or absence of blastodermal cells.

Chi-squared analyses of each cull-egg true fertility versus each control, settable egg true fertility combination revealed two significant comparisons. The pairwise comparisons using the true fertilities from the control, incubated treatment versus cull-thin and cull-cracked incubated eggs had *P*-values of 0.026 and 0.021, respectively. All pairwise comparisons with control-unincubated eggs were not statistically significant.

We conclude that normally formed but checked and cracked culled eggs can be used to determine true fertility by examination of the germinal disc and provide an estimate of the duration of fertility using the PL-sperm hole procedure. Such practices would eliminate the use of settable eggs and create a use for eggs that otherwise would be discarded. It is recommended that the culled eggs be evaluated before dehydration of the egg reaches a point where it is difficult to visualize an intact germinal disc and to isolate the PL.

The strong positive correlation between shell membrane/eggshell thickness and the stage of development of the blastoderm when culled eggs were broken out is in agreement with observations by Gupta and Bakst [3]. In contrast to the present study, Gupta and Bakst [3] established a similar conclusion with eggs manually removed from the oviduct or induced to oviposit. Using the stage of embryo development as an indicator of the time the egg mass was in the oviduct, we conclude that soft shell and membranous eggs were oviposited prematurely and not in the uterus for the duration of the ovulatory cycle.

CONCLUSIONS AND APPLICATIONS

1. We found that otherwise settable eggs with checks and cracks could be used to determine true fertility and PL-sperm hole numbers.
2. We recommend that soft-shelled or membrane-bound eggs not be used to determine true fertility.

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