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THE INFLUENCE OF THE AGE OF HEN ON EARLY EMBRYONIC MORTALITY, FREQUENCY AND TYPES OF CHROMOSOMAL ABERRATION IN LAYING HENS

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INTRODUCTION

Embryonic mortality in chickens is not uniformly distributed over the course of incubation. About 65% of embryonic mortality occurs in two phases: an early phase, with a peak at about day 4 of incubation, and a late phase, with a peak at about day 19 of incubation (Payne, 1919 in Jassim *et.al*, 1996). The frequency of early embryonic mortality has been observed to increase between 2 and 4 day incubation (Hutt and Pilkey, 1930 in Christensen, 2001). Christensen (2001) and Hocking and Bernard (2000) reported that the age of the dam affects early embryonic mortality. Flock age 33-35 weeks had significantly lower embryonic mortality than 53-55 weeks on day 3 incubation and a significant contributing factor in reducing hatchability (Deeming and Van Middelkoop, 1999).

Thorne *et al.* (1991) reported that chromosome abnormalities were responsible for 4.4 to 28.1% (average 11.8 %) and 7.4 to 25.0 % (average 13.4 %) of the early embryonic mortality in broiler and layers respectively. The embryonic mortality was caused by chromosomal aberrations to an extend of 25 % (Lodge *et al.*,1974) and 50 % (Szalay and Hidas, 1989).

The purpose of this study to find the relationships between the age of hen, frequency of early embryonic mortality, frequency and types of chromosomal aberrations in two pure lines (A and D) of a commercial brown layer breeding programme.

MATERIAL AND METHODS

Eggs of the two lines (A and D) were collected daily stored for a maximum of 7 days and incubated for 72 h under standard conditions. Sampling was done at three times of laying cycle. The age of hens at the time of sampling varied from 20-24, 26-28 and 61-64 weeks. Eggs were fertilised by artificial insemination.

Chromosome preparations were made using a technique adapted from Vagt and Saar (1986). After 72 h incubation, the eggs were opened at the blunt end to examine the contents by stereo microscope. Infertile eggs and early embryo mortality were discarded. Eggs with normal development or abnormally retarded in growth (included abortive development embryo - Thorne *et al.* 1991) embryos were injected with 0.2 ml of a 0.005% colchicine and incubated for 1 h (37.5°C). Blastodisc was removed and embryo was washed with Hanks solution

(Hanks: Aqua dest = 1:9) and incubated in hypotonic solution (FCS: Aqua dest =1:5; 37.5^{o}C; 25 min). Hypotonic solution was removed by Pasteur pipette and 3 ml of fixative (acetic acid : methanol = 1:3; 0^{o}C) were added. Samples were kept minimum 45 min prior to making slides. Slides were stained in Giemsa solution for around 12 min.

Slides were scanned carefully on low power and 5 to 20 clear metaphase spreads were then counted at x 1000 magnification. The 8 largest chromosomes were analysed. When chromosomal aberration were detected more than 20 metaphase were counted. To confirm euploid and aneuploid aberrations, their availability should be proved once and three times, respectively.

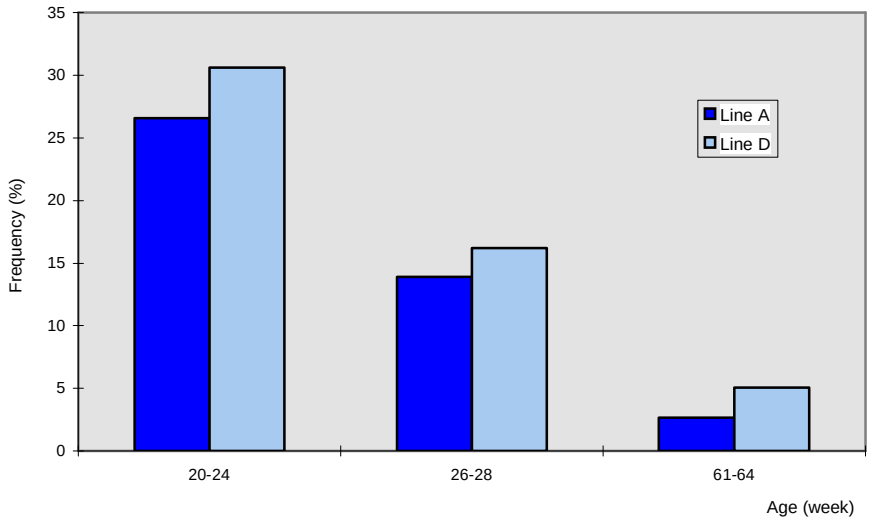
Contingency chi-squared test and Fischer exact test were used in the statistical analysis.

RESULT AND DISCUSSION

Relation between age of hen and frequency of early embryonic mortality.

Profile of early embryonic mortality in the lines A and D are presented in Figure 1. The Frequency of early embryonic mortality from young (20-24 weeks), mature (26-28 weeks) and old (61-64 weeks) hens were, respectively, 26.6%, 13.9% and 2.7 % in the line A, 30.6%, 16.2% and 5.1 % in the line D. In both lines it would be observed that frequencies of early embryonic mortality of young hens were higher than in old hens. The difference of frequencies between age of hens were significant ($P< 0.05$) and decrease with the age in both lines but the differences between lines were not significant. Hocking and Bernard (2000) reported that early embryonic mortality were higher in eggs from females aged 27 to 29 weeks compared with eggs from females aged 35 to 37 weeks. The factors associated with early embryonic mortality were discussed by Christensen (2001).

Fig. 1: Influence of age on frequency of early embryo mortality



Relation between age of hen and frequency of chromosomal aberrations.

Die effect of age of hen on the frequency of chromosomal aberrations are given in Table 1. The aberration frequencies in all age of hen were observed to vary from 8% to 14%. The line D was characterised by a higher frequency in all age of hen in the comparison to the line A. The differences between the lines and age of hen were statistically not significant. Chromosome aberrations found in various stocks ranged from 0.4 to 12.7% depending upon the strain, with higher value found in a meat-type rapid growth line (Bitgood and Shoffner, 1990). The frequency of aberration at the beginning of laying cycle in the boiler-type is reported relative high (Duber et al., 1973). In the ducks to the beginning of laying cycle were produced more aberrations than in the centre or at the end of laying cycle (Vagt and Saar, 1986). The values were 11.27% to the beginning of laying cycle and 4.20% in the end of laying cycle. But it is against the fact with quail that rising aberration frequencies showed in the laying cycle (from the beginning to the end) (Maarouf, 1988; Traore, 1999).

Table1: Comparison of aberration frequencies between Lines A and D

Line	Young	Mature	Old
A	9.4 % [(a)] (n=128)	8.0 % [(a)] (n=112)	10.2 % [(a)] (n=137)
D	13.0 % [(a)] (n=115)	14.0% [(a)] (n=107)	12.0 % [(a)] (n=158)

Differences are not significant between frequencies with same letter (P>0.05)

Relation between age of hen and types of chromosomal aberrations.

The distribution of types of chromosome alterations among each the age of hen are presented in Table 2. All of the chromosomal abnormalities in both lines are numerical aberrations. Most frequently in both lines and ages are euploid mosaics, which also was observed from several authors (Bloom, 1981; Szalay et.al., 1989; Thorne et al., 1991). With an exception in the line A for old hens pure haploidy is most frequent. The cause is individual amassment. Snyder et al., (1975) reported also that haploid abnormalities is not randomly distributed among dam. In both lines mature hens have fewer aberration types than young and old hens and in the young hens were also observed triploidy. The differences of the frequencies of the aberration types were significant only between mature and old hens for haploid type in the line A. Various forms of aberrations may arise as result of errors during spermatogenesis, oogenesis, fertilisation, or early cleavage division (Bloom, 1970; Snyder et al., 1975). These problems could be the result of genetic defects (Thorne et al., 1997) and a hormone imbalance (Jaap and Fechheimer, 1974).

Table 2: Frequencies of aberration types in the laying period of lines A and D

Types of aberration	Line A			Line D		
	Young	Mature	Old	Young	Mature	Old
Euploid	**3(25.0%); [2.3%]**	**1(11.1%); [0.9%](*)**	**8(57.14%); [5.8%](*)**	**3 (20.0%); [2.6%]**	-	**3 (15.8%); [1.9%]**
Haploid	1(8.3%); [0.8%]	1(11.1%); [0.9%]	7(50.0%); [5.1%]	1 (6.7%); [0.9%]	-	3 (15.79%); [1.9%]
Triploid	2(16.7%) [1.6%]	-	1(7.1%); [0.7%]	2 (13.3%); [1.7%]	-	-
Euploid mosaic	**6(50.0%); [4.7%]**	**7(77.8%); [6.3%]**	**3(21.4%); [2.2%]**	**11(73.3%); [9.6%]**	**10(66.7%) [9.4%]**	**12(63.2%); [7.6%]**
Haploid mosaic	5(41.7%); [3.9%]	7(77.8%); [6.3%]	3(21.4%); [2.2%]	9(60.0%); [7.8%]	10(66.7%); [9.4%]	8(42.1%); [5.1%]
Triploid mosaic	-	-	-	1(6.7%); [0.9%]	-	4(21.1%); [2.5%]
Tetraploid mosaic	1(8.3%); [0.8%]	-	-	1(6.7%); [0.9%]	-	-
Aneuploid mosaic	**2(16.7%); [1.6%]**	**1(11.1%); [0.5%]**	**3(21.4%); [2.2%]**	-	**4(26.7%); [3.7%]**	**4(21.1%); [2.5%]**
Trisomic mosaic	1(8.3%); [0.8%]	-	-	-	-	-
Monosomic mosaic	1(8.3%); [0.8%]	1(11.1%); [0.9%]	3(21.4%); [2.2%]	-	4(26.7%); [3.7%]	4(21.1%); [2.5%]
Mix form	**1(8.33%); [0.78%]**	-	-	**1(6.7%); [0.9%]**	**1(6.7%);** [0.9%]	-
Monosomic haploid mosaic	1(8.3%); [0.8%]	-	-	1(6.7%) ; [0.9%]	-	-
Double monosomic haploid mosaic	-	-	-	-	1(6.7%); [0.9%]	-

( ): from total of aberrations (*): significant different between frequencies

[ ]: from successfully analysed embryos

CONCLUSION

The study indicate a significant influence of the age of hen on the frequency of early embryonic mortality: young hens have a higher early embryonic mortality than old hens. There is no clear relationships between age of hen on the frequency of chromosomal aberrations but young hens (20-24 weeks) and old hens (61-64 weeks) have more types of chromosomal aberrations.

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