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Early Embryogenesis in Chicken Eggs of Different Weights[1]

K. L. Arora[2] and L. H. Matsumoto[3]

Abstract. Early embryogenesis in eggs of different weights, from a commercial strain of White Leghorn chickens, was studied. Observations were limited to unincubated blastoderms and embryos after 38 hours of incubation. Large eggs were found to be associated with high frequency of "degenerating" blastoderms as compared to medium and small eggs. Mean width of the unincubated blastoderms was directly related to the egg size. Such a direct relationship was, however, not so obvious in 38-hour embryos, probably because of a high degree of developmental variability associated wth large eggs. Embryonic development in medium sized eggs was consistently superior and invariably more uniform. The incidence of early embryonic mortality and abnormal embryogenesis was also higher in large eggs. These results were discussed in light of the observations from eggs subjected to prolonged preincubation storage.

It has been demonstrated repeatedly that over-all growth rate of the chick embryo, as well as the development of individual structures and organs, can be altered not only by hereditary factors but also by several environmental factors located either within the egg or acting through the outer environment. Among various factors frequently mentioned are: the genotype of the parents, age and nutrition of the dame laying the eggs, post-oviposital age of the egg, environmental conditions during preincubation storage and during incubation, and certain characteristics of the egg such as size, shape, and shell quality.

The influence of egg weight on hatchability and the growth of the embryo during the latter part of incubation has been investigated by numerous workers. It is now generally agreed that (a) the eggs which weigh considerably above or below the mean of the flock or of an individual hen hatch poorly, especially so at the higher egg weights, and (b) the weight of the embryo during the latter part of incubation or the weight of a newly hatched chick is proportional to the weight of the egg. The pertinent literature on this subject has been discussed by Bray and Iton (1); Kosin (2), and more recently in Landauer (3).

On the basis of the above mentioned egg size-embryo size relationship during the latter part of incubation, one is inclined to assume that such a relationship must have existed right from the beginning of development. Very little information is available in the literature on this subject, particularly relating to the development of embryos from

[1]Contribution No. 23, Department of Biology, Drake University, Des Moines, Iowa. The authors wish to express their appreciation to Hy-Line Poultry Farms, Johnston, Iowa, for making the material available to us for this study.

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eggs of different weights. The earliest report was that of Byerly (4) who suggested that the rate of development in the chicken embryos during early stages is probably independent of egg size. The more recent work of Krzanowska (5) and McNary et al. (6) has corroborated, in principle, these observations. On the other hand, McNally and Byerly (7) reported that, in general, the larger eggs had embryos at more advanced stages of development after 48 hours of incubation (as judged by the number of somites) while some small eggs contained embryos which had developed further than would be predicted from their weights. They further found that in eggs from individual hens, the number of somites increased with the increasing weight of the egg. Wiley (8) demonstrated from his histological observations with 72-hour chick embryos that the embryos from large eggs had a greater number of cells per unit area of embryonic tissues, where the embryos from small eggs were characterized by larger size of the individual cells in the tissues. However, any controlling effect of egg size on the rate of cellular proliferation and cell size diminished as incubation progressed. More recently, Resnjanskaja (9) offered convincing evidence that during the beginning of incubation, the embryonic growth rate was directly proportional to the egg weight.

The study reported here was undertaken to explore further this economically and basically important subject. The main objective of this study was to analyze the developmental potential of early chick embryos (blastoderms) contained in eggs of different weights. These observations are a by-product of studies underway in this laboratory investigating the role of heredity and environment on early embryogenesis.

Materials and Methods

The eggs utilized in this study came from Hy-Line commercial stock of White Leghorn birds. The eggs were gathered daily between 9 a.m. and 11 a.m. for two consecutive days. Soon after collection, the eggs were transferred to a holding room maintained at 13 ± 2° C. and 75 percent relative humidity. The eggs, after overnight cooling, were separated on the basis of their weights into three size categories: large (65-70 grams), medium (57-62 grams), and small (49-54 grams). Those eggs falling in between these size cateories were not utilized. Eggs of abnormal shapes or poor shell quality were also discarded.

Series 1. The eggs from each of these egg size categories were carefully opened into a fingerbowl containing warm saline solution (37° C.). The yolks, after separation from other egg contents, were transferred with the aid of a spoon, into another fingerbowl two-thirds filled with fresh saline solution. Here the yolk stood slightly above the level of saline solution with blastoderm uppermost. The blastoderms were then stained with a vital dye, Neutral Red, by placing a small strip of agar impregnated with stain on the vitelline membrane overlying the blasto-

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derm. After gross examination of the blastoderms, particularly with respect to the distribution of vacuoles, after the method of Arora and Kosin (10), the width of the blastoderms was measured with the aid of vernier calipers.

Series 2. Another set of eggs containing all size categories were incubated, with their blunt end upward at 37.5° C. and 65 percent relative humidity. Following 38 hours of incubation, a small window (approximately 1.5 centimeters square) was made in the blunt end of the eggs and the embryos were then stained, in-situ, with Neutral Red as described above. Following detailed morphological examination, measurements of different embryonic and extra-embryonic structures were made on each embryo according to the procedure outlined by Kosin and Arora (11).

Results

The data concerning the size of blastoderms is summarized in Table 1. It appears that the mean width of the unincubated blastoderm is directly related to egg size. Mean width of blastoderm was 4.11 mm., 3.78 mm., and 3.65 mm. in large, medium, and small eggs, respectively. Only the difference of 0.46 mm. between medium and small egg categories was statistically significant at the 5 percent level. In spite of individual variation, the range of distribution of blastodermal diameters showed that the proportion of the blastoderms with larger diameter is greater in large eggs as compared to those in medium and small eggs.

Table 1

Frequency (Percent) Distribution of the Blastoderm Diameter in Three Egg Size Categories

Egg Size	No. of Blastoderms	Mean Diameter (mm.)	Diameter of the Blastoderm (mm.)		
			3.6 or less	3.7 - 3.8	3.9 or more
Large	42	4.11	23.8 (10)	28.6 (12)	47.6 (20)
Medium	38	3.78	47.4 (18)	36.8 (14)	15.8 (6)
Small	46	3.65	47.8 (22)	34.8 (16)	17.4 (8)

Number of individuals are given in parentheses.

With regard to the size and distribution of vacuoles over the surface of the blastoderms or along the periphery of the area opaca, a wide individual variation was noticed. A total of 146 blastoderms from all three categories were examined. The frequency of blastoderms exhibiting varying degrees of "degenerative" changes was found to be 14.6 percent, 5.0 percent, and 4.2 percent in large, medium, and small eggs, respectively. Some blastoderms, particularly from large eggs, exhibited vacuoles of various sizes and shapes in the area opaca as well as in the area pellucida which, in advanced cases, gave the blastoderm a reticular

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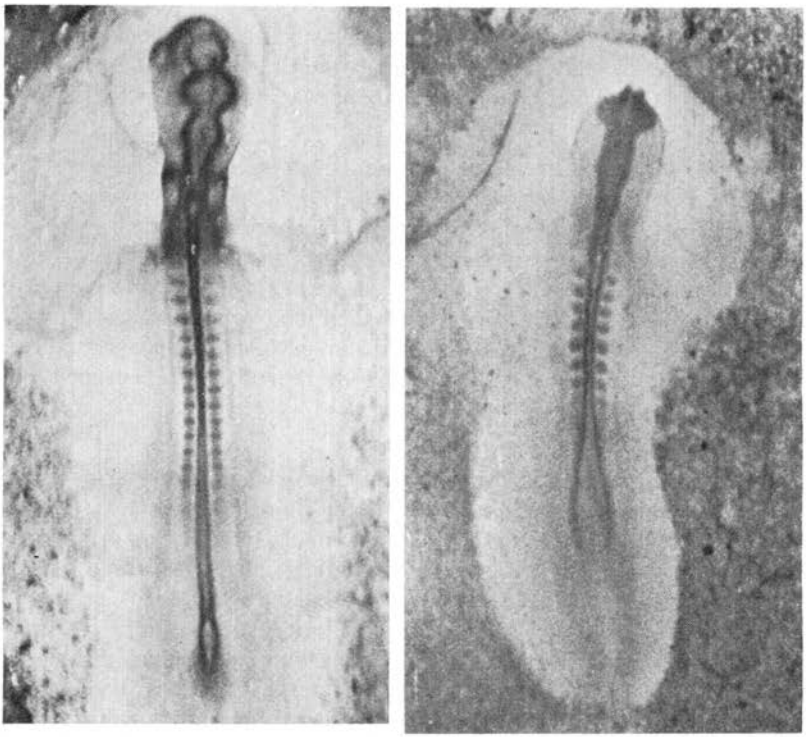

Figures 1-2. Variation in development of chick embryos in large eggs incubated for 38 hours. Embryos at 7 somite (Figure 2) and 14 somite stages (Figure 1).

appearance. On the basis of morphological examination it was impossible to distinguish such blastoderms from those exhibiting true biological infertility or pre-oviposital mortality.

Table 2 presents data on the development of several morphological traits in large, medium, and small eggs after 38 hours of incubation. Using length of the embryo, length of the area pellucida, number of somites, and width of one side of the area vasculosa as criteria, it was found that embryos in medium eggs were consistently superior in development to those from large and small eggs. In most cases, however, the differences were not statistically significant. In general, the rate of embryonic development in small eggs was comparatively lower than in large and medium eggs.

Considerable variation in the development of embryos was also noticed in this data. As shown in Table 2, the variability in the rate of embryonic development, as expressed in percent C. V., was the lowest in eggs of medium size; it was higher in small eggs; and it was the highest in large eggs. Some eggs in the latter category contained embryos at much more advanced stages of development than would

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Table 2

Early Embryonic Development in Eggs of Different Sizes.
38 hours of incubation

		Embryonic Traits							
		Number of Somites		Length of Embryo (mm.)		Length of Area Pellucida (mm.)		Width One Side of Area Vasculosa (mm.)	
Egg Size	n	Mean	% C.V.	Mean	% C.V.	Mean	% C.V.	Mean	% C.V.
Large	31	10.9	23.2	4.1	15.2	5.5	12.6	6.4	15.1
Medium	37	11.1	9.2	4.2	7.0	5.6	8.4	6.8	8.3
Small	36	9.9	11.3	3.6	9.4	5.0	11.2	6.1	10.5

C.V. = Coefficient of variation.

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be expected by 38 hours of incubation while some embryos exhibited retardation in their development. Out of 126 embryos examined, 21 embryos were either dead or exhibited abnormal development. As shown in Table 3, the frequency of early embryonic mortality is the highest in large eggs (about 13 percent of all developing embryos). Comparatively, the level of mortality was lower in medium (2.5 percent) and small eggs (5.0 percent). Table 3 further shows that the incidence of developmental abnormalities was about three times higher in large eggs than in medium and small eggs combined.

Table 3

Frequency (Percent) of Dead, and Abnormal Embryos in Eggs of Different Sizes. 38 hours of incubation[a]

Egg Size	n	Dead Embryos	Abnormal Emybros
Large	46	13.0 (6)	21.4 (9)
Medium	40	2.5 (1)	5.0 (2)
Small	40	5.0 (2)	2.5 (1)

[a]Number of individuals are given in the parentheses.

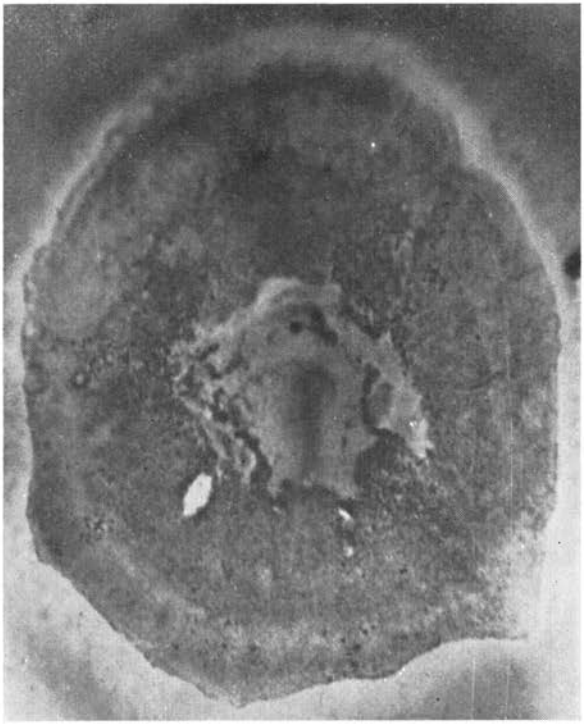

Figure 3. Retarded development. A blastoderm from a large egg exhibiting the [illegible] after 38 hours of incubation.

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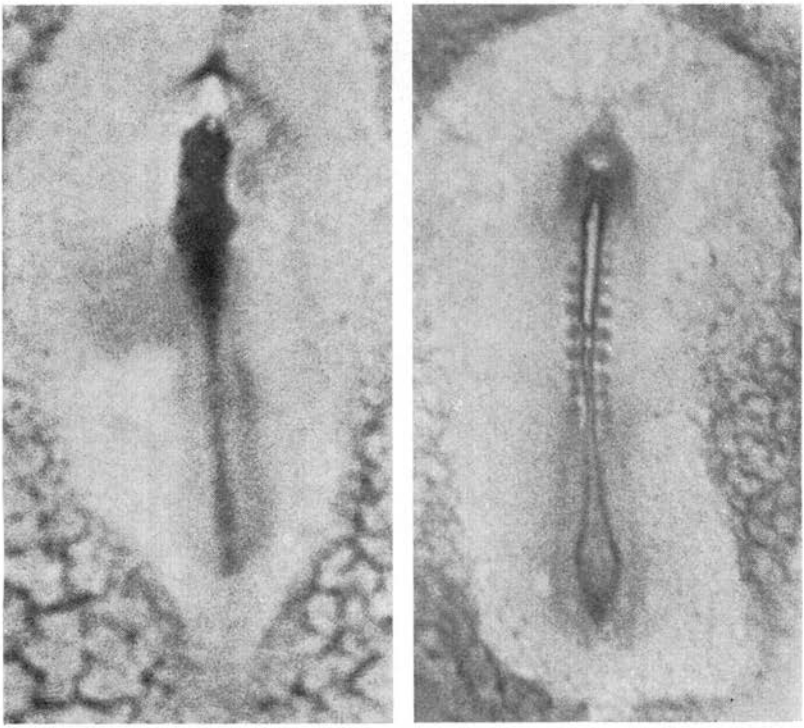

Figure 4. (left) Abnormal development. Absence of somites and poor development of the anterior region of the neural tube. Small egg. Figure 5. Abnormal development. Absence of the most anterior part (head region) of the neural tube. Large egg.

The following types of deformities were encountered: incomplete or irregular differentiation of the area pellucida, failure of neural tube to close, irregular pairing of somites, and twinning of embryos. During early incubation, some blastoderms failed to sustain growth and led to the formation of vacuoles all over their surfaces. In some cases, the embryonic tissue was found to have degenerated and extra-embryonic tissue continued to proliferate.

The statistical analysis of the effect of egg size is summarized in Table 4, indicating clearly that the treatments were effective in producing differences in early development, at least up to 38 hours of incubation. The mean square values for length of the embryo, length of the area pellucida, and width of one side of the area vasculosa are highly significant ($P < 0.01$). The mean square value for number of somites was significant only at the 5 percent level.

The correlation coefficient (r) among different embryonic and extra-embryonic traits was calculated. Only the data from medium size eggs were utilized for these calculations. Table 5 shows that the

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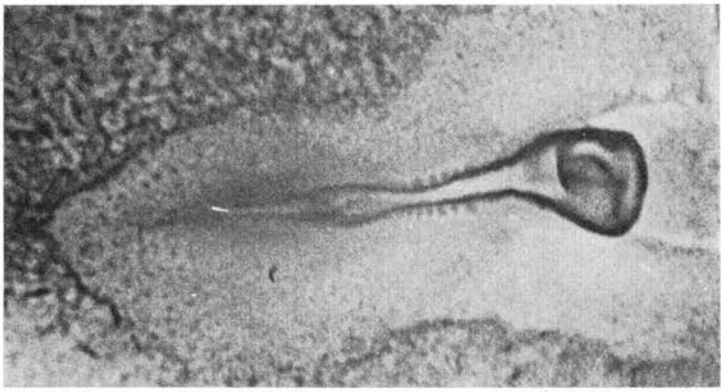

Figure 6. Abnormal development. Neural tube failed to close anteriorly. Large egg.

Table 4

Analysis of Variance of Several Embryonic Traits
38 hours of incubation

Sources of Variation	df	Mean Squares: Number of Somites	Length of Embryo	Length of Area Pellucida	Width of Area Vasculosa (one side)
Between groups (Egg-size)	2	13.6*	3.35**	3.0**	4.1**
Within groups	101	3.48	0.23	0.57	0.36
Total	103				

*Significant at 0.05 percent level.
**Significant at 0.01 percent level.

Table 5

Correlation Coefficients Among Several Traits in Chicken Embryos in Eggs of Medium Size.
38 hours of incubation[a]

Covariables	Coefficient of Correlation (r)
Number of somites and embryo length	.875**
Length of embryo and length of area pellucida	.784**
Length of embryo and width of area vasculosa (one side)	.545**
Number of somites and width of area vasculosa (one side)	.524**
Number of somites and length of area pellucida	.683**

**Significant at 0.01 percent level.
[a]Data given in this table was calculated from 37 individuals.

number of somites, length of the embryo, and length of the area pellucida are highly correlated traits. Correlation coefficient was, however, somewhat lower between length of the embryo and width of one side of the area vasculosa, and number of somites and width of one side of the area vasculosa.

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Discussion

Investigations concerning the influence of egg weight on early embryonic development is not only of interest to hatchery men, but also to biologists concerned with studying the norm of avian development and the factors affecting it. Important applied aspects of this problem lay in the fact that it may be possible to select for embryological studies or for haching those eggs which, because of their size, promise best results. Numerous workers have shown that large eggs hatch poorly, but the factors responsible for reducing hatching quality of such eggs have not been fully investigated. Besides genetic background, certain environmental conditions such as nutrition of the dame, composition of egg contents, quantitative relationship of albumin to yolk in the egg (12), certain physiological conditions of the oviduct (5), length of time spent by eggs in the oviduct (13, 7) have been suggested as possible contributing factors. Nutritional aspects of avian development have been reviewed recently by Taylor and Grau (14).

Whatever factors may be involved in the formation of large eggs and, indirectly for reducing the hatching quality of such eggs, it is the blastoderm whose developmental potential is really affected. With this in mind, the study reported here was designed to determine the effects of eggs' weight on the viability and developmental potential of the blastoderms. Examination of data gathered in this study revealed some interesting features regarding early embryogenesis. At oviposition, high proportions of blastoderms in large eggs were found to be associated with varying degrees of vacuolation ("degenerative" changes). The incidence of mortality and abnormal embryogenesis during early incubation was also much higher in large eggs in comparison to medium and small eggs. These findings may, at least in part, explain the poor hatchability of large eggs.

In spite of individual variation, the mean width of the blastoderm was directly related to egg size. Such a linear relationship was, however, not so obvious when the embryonic growth was compared after 38 hours of incubation. In general, the over-all growth of embryos in small eggs was consistently smaller than in medium and large eggs. Slightly lower mean values in large eggs was probably due to considerably higher degree of developmental variation associated with them, and the data from embryos expressing a wide range of deviations was pooled together. Some large eggs contained embryos at much more advanced stages of development than would be expected at this incubation age (38 hours of incubation). While some other embryos in large eggs exhibited marked retardation in development.

Because of a functional relationship which is known to exist between the stage of gastrulation at oviposition and the subsequent rate of embryogenesis in birds (15, 16, 11), it can be inferred, from the data obtained concerning 38-hour embryos, that there is considerable vari-

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ation among blastoderms in large eggs at the time of oviposition. Furthermore, the existence of moribund blastoderms in freshly-laid eggs, as well as embryonic mortality and malformation during early incubation, indicates some defective physiological state of grastrulae contained in the large eggs. Those observations and conclusions were quite similar to those reported previously (10, 17, 18, 19) with blastoderms subjected to extended periods of preincubation storage. Therefore, it can be said that the existence of certain "critical" stages of gastrulation and/or defective physiological state of gastrulae at the time of oviposition, makes them more sensitive to environmental conditions and may lead to failure in development, abnormal development, and death of the embryos during incubation.

Calculations of correlation coefficient among different embryonic and extra-embryonic traits indicated that the length of the embryo, length of the area pellucida, number of somites, and the width of one side of the area vasculosa are highly correlated. Any one of these traits or combination of these traits can be used effectively for comparing the rate of embryogenesis at this age of incubation.

Summary

Early embryogenesis was studied in eggs of different weights from a commercial strain of White Leghorn chickens. Eggs were separated on the basis of their weights, into three weight categories: Large (65 to 70 grams), medium (57 to 62 grams), and small (49 to 54 grams). Eggs of abnormal shapes or poor shell quality were discarded. Observations were made on unincubated blastoderms as well as on embryos after 38 hours of incubation at 37.5° C. and 65 percent relative humidity. Results indicate that:

1. The frequency of blastoderms exhibiting "degenerative" changes at the time of oviposition was about three times greater in large eggs as compared to medium and small eggs.
2. In spite of individual variation, the average width of the blastoderm was directly related to egg size.
3. The incidence of early embryonic mortality and abnormal embryogenesis was also much higher in eggs of large sizes.
4. Early embryonic growth was consistently superior and invariably more uniform in medium eggs than in large and small eggs. The embryos developing in large eggs exhibited a considerably higher degree of variation.
5. Length of the embryo, length of the area pellucida, number of somites, and width of one side of the area vasculosa are highly correlated traits during this stage of development.

The conclusions reached are that:

1. Certain "degenerative" changes at the cellular level in the blastoderm before oviposition may, in part, be responsible for the

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reduced viability of blastoderms and are expected to interfere with the sequential series of morphological processes during the incubation and possibly lead to early embryonic mortality, retardation in development, and abnormal embryogenesis.

2. In experiments where uniformity in embryonic growth is desired, the eggs of uniform and medium sizes should be utilized.
3. For comparing the rate of early embryogenesis, the quantitative measurement of any one or more of the following traits can be used effectively: length of the embryo, length of the area pellucida, and the number of somites.

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