University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Publications from USDA-ARS / UNL Faculty

U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska

2006

Comparative Staging of Embryo Development in Chicken, Turkey, Duck, Goose, Guinea Fowl, and Japanese Quail Assessed from Five Hours After Fertilization Through Seventy-Two Hours of Incubation

N. Sellier
Institut National de la Recherche Agronomique (INRA)

J.-P. Brillard
INRA

V. Dupuy
INRA

M. R. Bakst
USDA, murray@anri.barc.usda.gov

Follow this and additional works at: https://digitalcommons.unl.edu/usdaarsfacpub

 Part of the Agricultural Science Commons

Sellier, N.; Brillard, J.-P.; Dupuy, V.; and Bakst, M. R., "Comparative Staging of Embryo Development in Chicken, Turkey, Duck, Goose, Guinea Fowl, and Japanese Quail Assessed from Five Hours After Fertilization Through Seventy-Two Hours of Incubation" (2006). *Publications from USDA-ARS / UNL Faculty*. 629.
https://digitalcommons.unl.edu/usdaarsfacpub/629

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Publications from USDA-ARS / UNL Faculty by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

©2006 Poultry Science Association, Inc.
This article is a U.S. government work, and is not subject to copyright in the United States.

Comparative Staging of Embryo Development in Chicken, Turkey, Duck, Goose, Guinea Fowl, and Japanese Quail Assessed from Five Hours After Fertilization Through Seventy-Two Hours of Incubation

N. Sellier,*[1] J.-P. Brillard,† V. Dupuy,† and M. R. Bakst‡[2]

**Institut National de la Recherche Agronomique (INRA), Station Expérimentale des Palmipèdes à Foie Gras, Artiguères, 40280-Benquet, France; †INRA, Station de Recherches Avicoles, 37380-Nouzilly, France; and ‡Agricultural Research Service, USDA, Biotechnology and Germplasm Laboratory, Beltsville, MD 20705*

Primary Audience: Poultry Breeder and Hatchery Personnel, Poultry Scientists, Avian Embryologists

SUMMARY

Normal tables of chicken embryo development are used to define specific stages of morphogenetic progression from the first cleavage divisions through hatching. Although established for the turkey and Pekin duck, the application of the normal tables of chicken embryo development to other birds of commercial and research importance needs be examined. Chicken, turkey, Japanese quail, and Pekin duck blastoderms from oviductal eggs showed differences in the rate of development that were inversely correlated with egg size. Oviposited eggs from these and additional species (goose, Muscovy and mule ducks, and Guinea fowl) were examined after 24 to 72 h of storage and at 6-h intervals up to 72 h of incubation. There was variation in the developmental stages of the blastoderm at the time of oviposition between and within the species and strains examined. Although it is recognized that the temporal rate of development will differ between different species and strains, the external features of any embryo in any given stage will be nearly identical.

Key words: aves, embryonic development, incubation, fertilization, morphogenesis

2006 J. Appl. Poult. Res. 15:219–228

DESCRIPTION OF PROBLEM

General Information

One dilemma in comparing embryo development between poultry species is the absence of a common reference of sequential stages of morphogenetic development. To counter this problem, the normal table of embryogenesis was devised to accurately assess embryo development during the oviductal and incubation phases of embryogenesis (see [1] for a review). This table is not only important for research on the morphogenetic development of the avian embryo, but it is also useful for investigators in the poultry industry attempting to uncover the basis of fertility and hatchability problems.

[1]Currently located at the Station de Recherches Avicoles, INRA, Nouzilly, France.
[2]Corresponding author: murray@anri.barc.usda.gov

The ability to differentiate a live from a dead embryo or a fertilized from an unfertilized germinal disc (**GD**) is a prerequisite for determining whether the problem lies with fertility or embryo mortality. Unfortunately, the ability of the hatchery technicians and poultry scientists to differentiate a viable embryo from an early dead embryo from an unfertilized GD is questionable [2]. This differentiation is very important when performing fresh egg breakouts or candling breakouts. When eggs are candled, the clear eggs (i.e., those with no indication of embryo development) are all classified as unfertilized, and the percentage of fertilized eggs gives what is known as candling fertility. When the clear eggs are opened and examined (breakout) and the GD visually examined to differentiate an early dead embryo from an unfertilized GD, then true fertility can be established [3]. This practice requires trained technicians and, in the case of eggs examined within 24 h from the onset of incubation, a normal table as a reference to identify the actual stage of embryo development. The usefulness of clear-egg breakouts in the turkey industry was demonstrated by Krueger [4]. Krueger's data revealed the magnitude of early embryonic mortality of some commercial turkey breeder flocks, suggesting that early embryonic mortality can be an insidious problem.

Precision in identifying the status (fertilized vs. unfertilized and stage of embryonic development) of the GD is crucial in understanding the basis of hatch failures. In studies describing the effect of preincubation on long-term storage of turkey and broiler eggs, Fasenko et al. [5, 6] observed early embryo mortalities of 7.2% in turkeys and 6.2% in broiler chickens. Bakst and Akuffo [2] reported that when the GD is critically examined at breakout, eggs from 18 turkey hens had 100% true fertility, 17 hens had 1 or more unfertilized or early dead embryos, and, of the 10 hens producing 1 or more early dead embryos, 3 hens were responsible for 50% of the early deads.

Notwithstanding their commercial importance, reports addressing early embryonic mortality and hatchability in ducks, geese, Japanese quail, and Guinea fowl are limited. Brun et al. [7] found no differences in early embryo mortality (2 to 3%) between mule (a commercial cross between a male Muscovy and female Pekin), Pekin, and Muscovy embryos at candling after 6 d of incubation. However, breaking out the clear eggs revealed an early embryonic mortality of 14.9% in the mules and 10.3% in the Pekin and Muscovy eggs [7].

Staging Embryos and the Normal Tables

The Hamburger and Hamilton (**HH**) procedure describing the progressive stages of embryo development during incubation is the most widely used normal table [8]. This staging procedure sequentially categorized the morphogenetic development of the chicken embryo from oviposition through hatching in 45 morphologically discrete stages. It was 25 yr after the HH staging was introduced that embryo staging prior to oviposition was systematically described. In their work, Eyal-Giladi and Kochav (**EGK**) [9] devised a 14-stage classification of the morphogenetic development of the early chicken embryo during the preoviposition, oviductal period. The stage XIV (EGK) blastoderm, which signifies the completion of hypoblast formation, coincides with stage 1 of HH. Staging procedures similar to the EGK for the chicken have been described by Gupta and Bakst [10] for the turkey and Dupuy et al. [11] for the Pekin duck.

Driven by the need to have objective assessments of embryo development in other species of commercial importance, several investigators compared the embryonic development of the Japanese quail, turkey, and duck to the chicken. Stepkinska and Olzanska [12] applied the EGK and HH staging procedures when comparing the development of chicken and Japanese quail embryos from first cleavage through hatching. Stepkinska and Olzanska [12] reported that Japanese quail and chicken embryos are morphologically comparable in their development but that the blastoderm in the fresh laid Japanese quail egg is slightly more advanced with hypoblast formation clearly evident. Turkey embryo development from first cleavage through hypoblast formation exhibited temporal and morphogenetic differences compared with the chicken embryo [10, 13]. These authors found that the chicken is further along in development, often at the onset of hypoblast formation, at the time of

oviposition, whereas the turkey egg must be incubated for the onset of hypoblast formation. Dupuy et al. [11] showed that the early morphogenetic development of the Pekin duck is morphologically comparable with the chicken's development but is less developed than the chicken embryo at oviposition. They also noted that a well-defined Koller's sickle is observed only in chicken embryos.

Why Stage Embryos?

Only with the detailed descriptions of the normal course of morphogenetic development of the avian embryo provided in normal tables can we obtain accurate, consistent, and repeatable data among laboratories. These have been done with the chicken, turkey, and duck embryos, and to a lesser extent with the Japanese quail embryo. This knowledge can be applied to activities such as defining normal vs. abnormal embryo development; in fresh egg breakouts to differentiate the early dead, from a fertilized ovum, from an unfertilized GD; to evaluate the effect of egg handling and egg-storage conditions on preincubation development; to evaluate the impact of hen age, strain, oviposition time, and shell quality relative to blastoderm development after oviposition, egg storage, and incubation; and to determine the comparative role and function of the morphogenetic processes on further embryonic development and survival. Although staging can be done by any trained individual, it is usually left to the research laboratory personnel and not hatchery personnel, primarily due to the substantial time investment in the preparation of each embryo.

As already noted, early embryo mortality is a problem in the poultry industry, and fresh egg breakouts are increasingly being used to estimate true fertility. Therefore, it would be helpful to have descriptions of the embryos from eggs stored up to 72 h from several domestic birds of commercial and research importance. Our primary aim was to compare and contrast the morphology of blastoderms isolated from the ova from chicken, Muscovy duck, Pekin duck, mule duck, turkey, Japanese quail, goose, and Guinea fowl 5 to 7 h after ovulation through 72 h of incubation. In doing so, embryos from all species were compared with the normal tables of the chicken as described by EGK and HH.

MATERIALS AND METHODS

Birds and Eggs

We individually caged a total of 214 Pekin duck females (8 wk of age) that were derived from an experimental line (**EXP**) and selected on the basis of the duration of their fertile period. These birds were subjected to 8L:16D up to 16 wk of age and then the light was progressively increased to a 14L:10D photoperiod for the laying period. A standard commercial feed was provided ad libitum to 8 wk of age, quantitatively restricted (80% from ad libitum) from 9 wk up to the onset of lay, and then ad libitum from peak of lay to the end of the experiment. All Pekin females were artificially inseminated with 120×10^6 sperm twice a week. Pooled semen from Pekin drakes was used to obtain fertilized EXP Pekin eggs, and pooled semen from Muscovy drakes was used to obtain fertilized EXP mule eggs.

Oviductal Eggs

To collect oviductal eggs from ducks, EXP Pekin females were euthanized with a lethal injection of pentobarbital immediately after oviposition and 5 to 7, 8 to 10, 11 to 13, 14 to 16, 17 to 19, 20 to 22, and 23 to 25 h after oviposition. Six to 10 oviductal eggs were collected per time period for a total of 70 eggs being examined.

Fresh and Incubated Eggs

Eggs from the 144 remaining Pekin females were collected daily from the second day after the first artificial insemination up to the seventh day after the last artificial insemination. In addition, fresh Pekin duck eggs were obtained from various commercial operations in France [14]. This provided 888 eggs for observations of embryo development during the first 72 h of incubation. In addition, 251 Landaise goose, 571 mulard (commercial strain), 521 Muscovy, 524 Guinea fowl, 563 Japanese quail, and 80 turkey eggs for staging and incubation were obtained from the Institut National de la Recherche Agronomique (INRA) research facilities (Nouzilly and Benquet, France). Additional chicken,

Table 1. Incubation conditions

Strain or breed	Temperature (°C)	Relative humidity (%)
Muscovy	37.6	55
Turkey	37.7	58
Mulard (EXP[1] + commercial)	37.6	60
Guinea fowl	37.7	57
Pekin (EXP)	37.6	65
Gallus	37.8	45
Goose	37.7	55
Japanese quail	37.8	47

[1]EXP = experimental line used by Institut National de la Recherche Agronomique (INRA), Nouzilly, France.

turkey eggs (Large White) and Japanese quail eggs were obtained from the Agricultural Research Service (USDA, Beltsville, MD) research farm and Japanese quail breeding colony at the University of Maryland-College Park.

Storage and Incubation Conditions

Eggs of all species were picked up daily and stored (15 to 18°C) for not longer than 3 d before incubation. Incubation in each species was performed according the standard conditions used in our laboratories (Table 1). Egg turning was performed once per hour for all species.

Preparations of Blastoderms

Eggs were broken out, and blastoderms were isolated and staged according to the EGK procedure. The isolation of the blastoderm and the removal of adhering yolk are described elsewhere [9, 10, 15]. Eggshells were cut transversely with scissors, and most of the remaining albumen was removed by tipping. The yolk was gently rotated to bring the GD into view, and the remaining albumen was removed gently with blotting paper. A precut filter-paper ring (inside diameter of 5 mm and outside diameter of 10 mm) was then grasped with fine forceps and placed on the yolk's surface with the GD in its center. The perivitelline layer was cut with iris scissors along the outer edge of the filter ring, and the filter ring with adhering GD was carefully lifted from the yolk surface and immersed in Ringer's saline. Adhering yolk was removed from the blastoderm with a fine hair loop and by blowing streams of Ringer's saline with a Pasteur pipette until the ventral surface of the GD was exposed. Further examination of the blastoderm was performed by stereomicroscopy [16] equipped with a digital camera [17].

Statistical Analyses

In the present study, the heterogeneity of variances between embryos from different genetic origins at every stage examined did not allow ANOVA (linear model) or nonparametric test. As a consequence, analyses were performed at oviposition by comparing staging of each species with chicken using a Proc Freq analysis [18] and 2×2 chi-squared test with the option of Fisher's exact test.

RESULTS

Oviductal Eggs and Eggs Incubated Through 72 h

Eggs were staged by methods described by EGK (stage in Roman numerals) and, if incubated, by the EGK and HH (stage in Arabic numerals) staging procedures. Pekin duck embryos were also staged with the procedure described by Dupuy et al. [11] (Arabic numerals plus D). Table 2 compares the progressive morphogenetic development of the fertilized GD while the egg mass was in the oviduct. At 14 to 16 h after fertilization, the morphogenetic development of the Japanese quail is the most advanced (stages VII to VIII) among the species examined. This is further accentuated at oviposition when the Japanese quail blastoderm is at stage XI in contrast to the chicken blastoderm at stage X ($P < 0.001$). Significant differences between the chicken blastoderm and all of the other species examined at oviposition were also observed ($P < 0.001$). The commercial and experimental strains of Pekin duck showed minor variation between their stages of development.

Table 3 contains the comparative morphogenetic development up to 72 h of incubated embryos according to the EGK and HH staging procedures plus the staging procedure described by Dupuy et al. [11] for Pekin ducks. The range of morphogenetic development

Table 2. Comparative development of the blastoderm[1] during passage of the egg mass though the oviduct using embryo staging procedures described by Eyal-Giladi and Kochav (Roman numerals; [9]) for chickens and Dupuy et al. (D plus Arabic numerals; [11]) for 2 strains of Pekin ducks.

	Hours after fertilization							
Strain or breed	5 to 7	8 to 10	11 to 13	14 to 16	17 to 19	20 to 22	23 to 25	Oviposition
Chicken[2]	I-II	III	IV-V	VI	VII	VIII-IX	IX-X	X
Turkey[2]	I-II	II-III	III-IV	IV	V-VI	VI	VI-VII	VII-VIII
Japanese quail[2]	I-II	II-IV	V	VII-VIII	VIII	IX	X	XI
Pekin (commercial)	I, 1D	I, 1D	III, 3D	V, 5D	VI, 6D	VII, 7D	VII, 7D	VIII, 8D
EXP Pekin[3]	I, 1D	II, 2D	III, 3D	VI, 6D	VI, 6D	VII, 7D	VIII, 8D	VIII, 8D

[1]Six to 10 oviductal egg masses were collected per time period and assessed.
[2]The developmental stages of the chicken [9], turkey [10], and Japanese quail [12] were cited for comparative purposes.
[3]EXP = experimental line used by Institut National de la Recherche Agronomique (INRA), Nouzilly, France.

within each 6-h interval between the species and strain examined was evident.

Embryo Staging at Oviposition

Within-species and strain variation was evident when eggs from the 0-time interval (24 to 72 h after storage) were broken out, examined, and staged. Although the chicken and Muscovy blastoderms showed the least amount of developmental variation, the Japanese quail blastoderm ranged from stage VI through stage XIV, with majority of blastoderms at stages XI to XII. Overall, Japanese quail and goose blastoderms were more advanced (stage XI) than the chicken (Stage X) while the ducks, Guinea fowl and turkey blastoderms were the least developed (stages VII to VIII). The range of developmental stages within the stored, unincubated eggs for each species and strain examined is presented in Table 4. At oviposition, the frequency of stage X embryos was significantly higher in chicken than in any other species ($P < 0.01$).

Chicken. The stage X chicken blastoderm consisted of an inner area pellucida and a dense outer ring, the area opaca (Figure 1A). The mottled appearance of the area pellucida was due to isolated cell aggregates on the ventral surface of the blastoderm. The stage XI blastoderm (Figure 1B) was characterized by a more conspicuous appearance of the isolated cell aggregates and the onset of hypoblast formation.

Goose. The goose blastoderm at stage XI was similar to that of the Japanese quail before incubation (Table 4). Some degree of hypoblast formation, stages XI to XII, was observed in about 50% of the blastoderms.

Japanese Quail. Of all the species examined the Japanese quail egg was morphogenetically the most advanced with about 65% of the blastoderms clearly in Stages XI to XII, the hypoblast stage of development.

Turkey. The turkey blastoderm at oviposition was equivalent to stage IX or early stage X. In contrast to the chicken, Kohler's sickle was not apparent in the late stage X blastoderms; however, the area alba, a centrally located aggregation of large blastodermal cells, was often present prior to incubation.

Mulard. These blastoderms were slightly less advanced than their maternal genetic counterpart (Pekin). They were identified as equivalent to stage VIII (Figure 1C). The area pellucida also was less lucent than that of the chicken, suggesting incomplete cell shedding. Consequently the area opaca appeared more voluminous than that of the chicken blastoderm at oviposition.

Guinea fowl. The majority of Guinea fowl blastoderms (Figure 1D) were at stage VII or VIII (Table 4).

Muscovy. Of all the blastoderms examined, the Muscovy duck (Figure 1E) had the least-developed appearance with the majority of blastoderms in stage VII (Table 4). Neither a definitive area pellucida nor the area opaca could be differentiated.

Pekin. Blastoderms in this species were observed in a range of developmental stages closely resembling the rate of morphogenetic

Table 3. Comparative embryo development during the first 72 h of incubation using normal tables by Eyal-Giladi and Kochav (Roman numerals; [9]), Hamburger and Hamilton (Arabic numerals; [8]) and Dupuy et al. (Arabic numerals plus D; [11]) for Pekin ducks.

Eggs examined (n)	Strain or breed	Incubation duration (h)													Hatch day
		0	6	12	18	24	30	36	42	48	54	60	66	72	
		(Embryonic stage)													
145	Chicken	X	2.0	3.0	4.0	6.0	9.0	10.0	11.0	12.0	16.0	17.0	18.0	20.0	21
251	Goose	XI**	XIII	2.0	4.0	5.0	6.0	8.0	11.0	12.0	13.0	14.0	15.0	16.0	28–30
524	Muscovy duck	VII**	X	XI	XIII	XIV	2.0	3.0	5.0	5.0	8.0	10.0	12.0	12.0	35–37
285	Mulard duck (commercial)	VIII**	X	XII	2.0	3.0	4.0	5.0	7.0	8.0	10.0	12.0	13.0	14.0	
286	Mulard duck (EXP)[1]	VIII**	XI	XIII	2.0	3.0	4.0	5.0	6.0	9.0	9.0	12.0	12.0	13.0	
396	Pekin duck (EXP)[1]	8D, VIII**	11D, XI	13D, XIII	16D, 3	17D, 4	18D, 5	20D, 7	22D, 9	24D, 11	26D, 13	27D, 14	28D, 15	28D, 15	28
492	Pekin duck (commercial)	8D, VIII**	11D, XI	13D, XIII	16D, 3	17D, 4	19D, 6	21D, 8	22D, 9	24D, 11	25D, 12	27D, 14	29D, 16	30D, 17	28
563	Guinea fowl	VIII**	XI	XII	4.0	3.0	4.0	6.0	8.0	9.0	11.0	11.0	13.0	15.0	28
142	Japanese quail	XI**	XIII	3.0	4.0	6.0	9.0	12.0	13.0	14.0	15.0	17.0	17.0	19.0	16–18
80	Turkey	VIII**	VII		X	XII		5.0		7.0			10.0		26

[1]Experimental lines used by Institut National de la Recherche Agronomique (INRA).
**Within column 0 (oviposition), data from a given species were compared with observations performed on the chicken (chi-squared test). Values with superscripts different from chicken were highly significantly different from that species ($P < 0.01$).

Table 4. The distribution (%) of developmental stages (Eyal-Giladi and Kochav) of embryos from oviposited eggs stored up to 72 h

Stage	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Chicken	0	0	0	0	4.2	83.3	12.5	0	0	0
Goose	0	0	0	11.1	0	27.7**	22.2	27.7**	11.1**	0
Muscovy duck	0	19.0*	80.9**	0	0	0**	0	0	0	0
Mulard duck (commercial)	0	7.4	37.0	33.3	18.5	3.7**	0	0	0	0
Mulard duck (EXP)[1]	0	5.9	37.2**	43.1**	5.9	2.0**	3.9	0	2.0	0
Pekin duck (EXP)[1]	2.3	2.3	22.7**	34.0**	20.4	18.2**	0*	0	0	0
Guinea	0	0	35.0*	57.5**	5.0	2.5**	0*	0	0	0
Japanese quail	0	2.3	2.3	6.8	4.5	11.4**	40.9*	25.0**	4.5	2.3
Turkey[2]	0	0	0	4.4	53.5	40.3**	1.7**	0	0	0

[1]Experimental lines (EXP) used by the Institut National de la Recherche Agronomique (INRA).
[2]Turkey embryos were staged at the Agricultural Research Service (ARS, USDA, Washington, DC). All other staging was performed at INRA.
*,**Within each column, data from a given species were compared with observations performed in the chicken (chi-squared test); *significantly different ($P < 0.05$) and **highly significantly different ($P < 0.01$).

development of other duck embryo strains and Guinea fowl.

Unfertilized GD at Oviposition

Regardless of species or strain, unfertilized GD from oviposited eggs stored no longer than 72 h had similar characteristics. The GD was most often dense white and asymmetrical and contained varying numbers of vacuoles scattered in the periphery or distributed randomly throughout the GD (Figure 1F). Vacuoles varied in size, some being grossly visible, but most were too small to discern without magnification.

DISCUSSION

Bellairs and Osmond [1] noted that normal tables "...enable the developmental state of an embryo to be assessed and communicated relatively accurately." They further went on to describe potential pitfalls one encounters when using normal tables of development. A questionable situation we have encountered is the applicability of normal tables of chicken development to other avian embryos. In this study, the morphogenetic development was staged for blastoderms from several species and strains of birds of economic and research importance. Our examination revealed subtle differences in the relative rates of embryo development from lay through 72 h of incubation between species and strains. In addition, breakouts of eggs from individual species and strains before incubation revealed considerable variation in the stage of blastoderm development within species and strains.

In the species examined, the initial cleavage furrows were observed about 5 to 7 h after fertilization, which suggested that the events surrounding fertilization and leading to cleavage furrow formation were temporally similar in the birds examined. By 8 to 10 h after fertilization, differences in the rate of morphogenesis were becoming apparent, and the Japanese quail was developing faster than the chicken, the chicken faster than the turkey, and the Pekin duck strains at approximately the same rate as the turkey.

There appears to be a general, but not exclusive, tendency that incubation times are positively correlated with egg size [19]. However, as shown in this study, egg size has minor association with the stage of embryo development, at least up to 72 h of incubation. When the eggs were broken out, morphogenetic development was least advanced in the Muscovy duck and guinea fowl (stage VII), and their area pellucidae were barely discernible. Alternatively, with the onset of hypoblast formation, the goose and Japanese quail (Stage XI) blastoderms were the most developed. The physiological bases for such morphogenetic differences may be collectively described as being genetically controlled. One would suspect that the species with the shortest incubation period would have the most morphogenetically developed blastoderm at oviposition. Although this

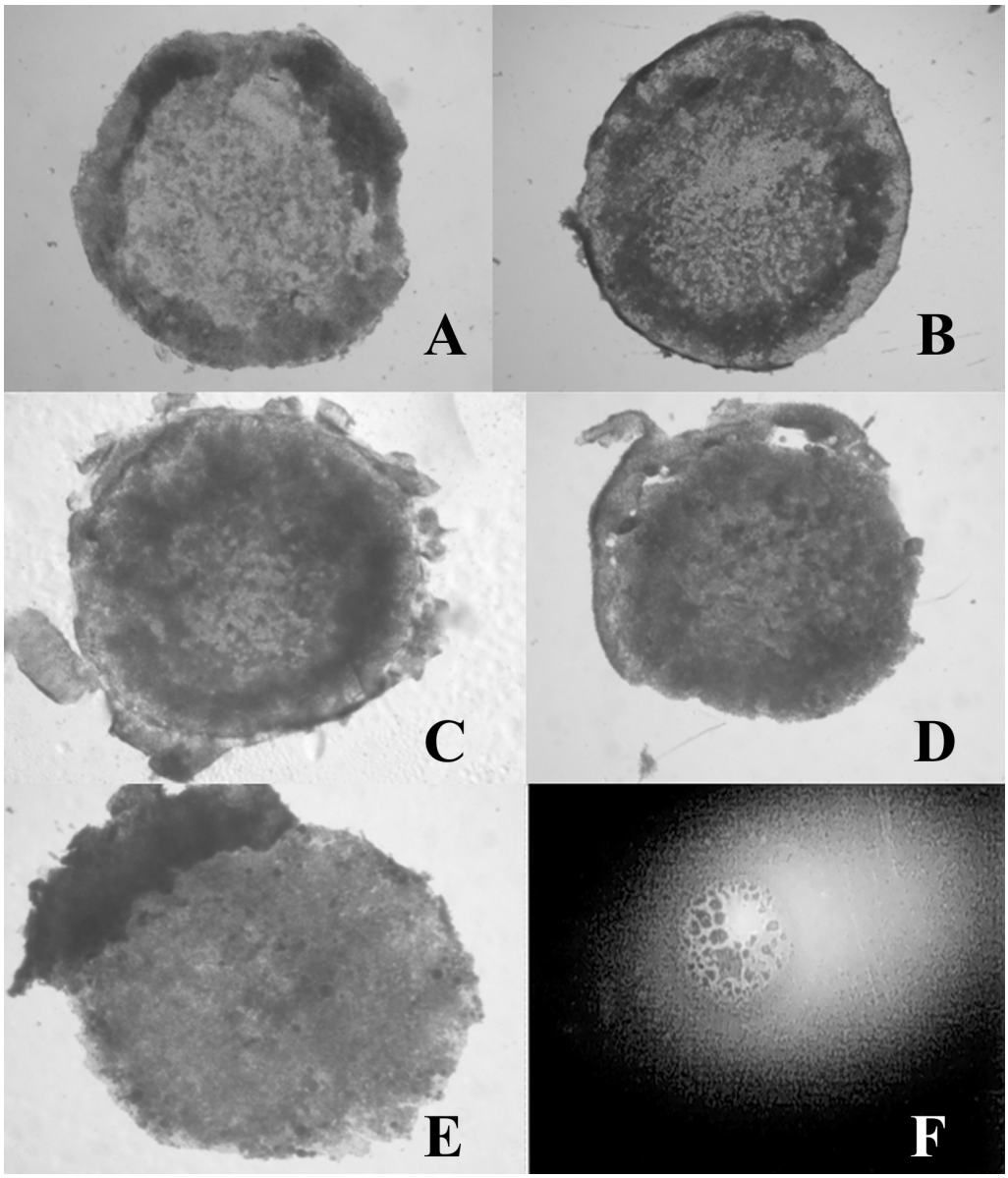


Figure 1. Panel A: A 4.5-mm diameter stage X chicken blastoderm is characterized by a dense ring, the area opaca, enveloping the less central region, area pellucida. The mottled surface of the area pellucida is due to aggregates of cells.
Panel B: A 4.5-mm diameter stage XI chicken blastoderm has the same characteristics as the stage X blastoderm, except that the aggregates of cells appear denser, and one side of the area pellucida appears denser due to the forming hypoblast.
Panel C: This 4-mm diameter stage VIII mulard blastoderm shows incomplete area pellucida formation and a relatively wide area opaca.
Panel D: This 4-mm diameter late stage VII Guinea fowl blastoderm shows the beginning of the area pellucida and area opaca formation.
Panel E: This stage VI Muscovy duck blastoderm shows no sign of area pellucida formation and is nearly uniform in its 3.5-mm diameter.
Panel F: This turkey's unfertilized 3.5-mm diameter germinal disc, which is representative of most species and strains examined, is recognized by its dense white appearance and is accompanied by a variable number of vacuoles in the white yolk area. When viewed without magnification, the dense white area appears eccentric and asymmetrical due to the random distribution and density of the vacuoles.

appears to be a satisfactory explanation for the Japanese quail (stage XI; 16 to 18 d incubation) and broiler chicken (stage X; 21 d incubation), this suggestion loses some validity when blastoderms of guinea fowl (stage VII; 28 d incubation), turkeys (stage X; 28 d incubation), and mulard (stage IX; 30 d incubation) are considered. An association between stage of blastoderm development at the onset of incubation and the length of incubation, therefore, seems unlikely. However, the stage of development at the time of incubation may influence hatching success. Prewarming of chicken or turkey eggs for 6 or 7 h, respectively, advanced blastoderm development by 2 to 4 stages and resulted in increased hatchability of stored eggs [5, 6].

Other factors that may account for variations in blastoderm development among species examined here may include strain origin, time variation in the oviductal period of blastoderm development, postlay egg management techniques, and degree of domestication (selection pressure may have inadvertently selected for or against factors in the GD affecting embryogenesis). The least variation in the development of the blastoderm at oviposition was with the chicken, arguably the most domesticated of the species and strains examined.

A uniform staging procedure combining the HH with the EGK normal tables has been suggested by Dupuy et al. [11]. However, this is based on the Pekin duck and only goes to 72 h of incubation. Until a single and uniform staging procedure based on the morphogenetic development of the chicken is developed, we suggest that the normal tables of chicken embryo development devised by Eyal-Giladi and Kochav [9] for oviductal eggs and Hamburger and Hamilton [8] for incubated eggs be applied to the morphogenetic development of the species and strains examined in this study. Although we recognize that the temporal rate of development differs among different species, strains, and even individuals, the external features of any embryo within any given stage will be nearly identical.

CONCLUSIONS AND APPLICATIONS

1. The normal tables of chicken embryo development devised by Eyal-Giladi and Kochav for oviductal eggs and Hamburger and Hamilton for incubated eggs are applicable to embryos of other commercial and research bird species.
2. Investigators using the normal tables of chicken embryo development to describe embryo differentiation in other bird species should expect to observe temporal differences in the appearance of specific stages of development.

REFERENCES AND NOTES

1. Bellairs, R., and M. Osmond. 1998. The Atlas of Chick Development. Acad. Press, New York, NY.

2. Bakst, M. R., and V. Akuffo. 2002. Impact of egg storage on embryo development. Avian Poult. Biol. Rev 13:125–131.

3. Bakst, M. R., S. K. Gupta, W. Potts, and V. Akuffo. 1998. Gross appearance of the turkey blastoderm at oviposition. Poult. Sci. 77:1228–1233.

4. Krueger, K. K. 1990. Fertility in female turkeys: How to manage it? Pages 205–212 in Control of Fertility in Domestic Birds. J. P. Brillard, ed. INRA, Nouzilly, France.

5. Fasenko, G. M., V. L. Christensen, M. J. Wineland, and J. N. Petitte. 2001. Examining the effects of prestorage incubation of turkey breeder eggs on embryonic development and hatchability of eggs stored for four or fourteen days. Poult. Sci. 80:132–138.

6. Fasenko, G. M., F. E. Robinson, A. I. Whelan, K. M. Kremeniuk, and J. A. Walker. 2001. Prestorage incubation of long-term stored broiler breeder eggs: 1. Effects on hatchability. Poult. Sci. 80:1406–1411.

7. Brun, J. M., N. Sellier, M. M. Richard, F. Batellier, and J. P. Brillard. 2004. Effect of in vivo sperm storage and breeder hen's age on fertility and embryonic survival in pure Pekin duck hens and their crossbreeding with Muscovy drakes. Proc. XXII World's Poult. Congr., Istanbul, Turkey.

8. Hamburger, V., and H. L. Hamilton. 1951. A series of normal stages in the development of the chick embryo. J. Morphol. 88:49–92.

9. Eyal-Giladi, H., and S. Kochav. 1976. From cleavage to primitive streak formation: Complementary normal table and a new look at first stages of the development of the chick. I. General morphology. Dev. Biol. 49:321–337.

10. Gupta, S. K., and M. R. Bakst. 1993. Turkey embryo staging from cleavage through hypoblast formation. J. Morphol. 217:313–325.

11. Dupuy, V., B. Nersessian, and M. R. Bakst. 2002. Embryonic development from first cleavage through seventy-two hours incubation in two strains of Pekin duck (*Anas platyrhynchos*). Poult. Sci. 81:860–868.

12. Stepinska, U., and B. Olsanska. 1983. Cell multiplication and blastoderm development in relation to egg envelope formation during uterine development of Japanese quail (*Coturnix coturnix japonica*) embryo. J. Exp. Zool. 228:505–510.

13. Bakst, M. R., S. Gupta, and V. Akuffo. 1997. Comparative development of the turkey and chicken embryo from cleavage through hypoblast formation. Poult. Sci. 76:83–90.

14. Pekin ducks, Grimaud Frères Sélection, Roussay, France.

15. Bakst, M. R. and H. Cecil. 1997. Techniques for Semen Evaluation, Semen Storage, and Fertility Determination. Poult. Sci. Assoc., Savoy, IL.

16. Stemi SV11 stereomicroscope, Carl Zeiss SA, Le Pecq, France.

17. Photomicrographs were obtained with a 4.0 megapixel Powershot G3 Canon Digital Camera, Canon SA, La Garenne Colombes, France.

18. SAS Institute. 1999. SAS/STAT User's Guide, Version 8. SAS Institute Inc, Cary, NC.

19. Wilson, H. R. 1991. Effect of egg size on hatchability, chick size, and posthatching growth. Pages 279–283 in Avian Incubation. S. G. Tullett, ed. Butterworth-Heinemann, Elsevier Books, Oxford, UK.

Acknowledgments

This study was conducted with the financial support of Region Aquitaine. The authors thank V. Akuffo (Agricultural Research Service, USDA, Biotechnology and Germplasm Laboratory, Beltsville, MD) and C. St-Cricq, M. Lague, and L. Million (INRA, Station Expérimentale des Palmipèdes à Foie Gras, Artiguères, France) for their technical assistance.