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Gross Appearance of the Turkey Blastoderm at Oviposition

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ABSTRACT The blastoderm (fertilized ovum) and unfertilized germinal disc (UGD) of fresh laid eggs and eggs stored prior to incubation exhibit subtle but definable morphological variations. Such variations may lead to difficulty when attempting to determine true flock fertility based on the appearance of the blastoderm / UGD. The objectives of this study were to define and categorize such morphological variations and to determine whether sperm influence the frequency distribution of the different categories. Eleven categories of blastoderms were defined based on the relative density and appearance of the area alba, area pellucida, area opaca, and the periblast. The majority of the blastoderms were included in the first four categories. Unfertilized germinal discs were divided into six categories and were best differentiated from the blastoderms by the presence of vacuoles around its central dense area. They were also discernible from blastoderms based on their overall denser appearance. Differences in the frequency distribution of some of the UGD categories between virgin and inseminated hens may be due to the effect that supernumary sperm may have on the organization of the UGD (no fertilization but supernumary sperm present) or blastoderm (fertilized but failed to develop). It is recommended that before starting true fertility determinations during fresh egg breakouts, one should study the appearance of the UGD from virgin hens and then the blastoderm from inseminated hens. One then will learn to appreciate the subtle differences in shape and density of the blastoderm / UGD structural components.

(*Key words*: true fertility, hatchability, infertile germinal disc, blastoderm, turkey)

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INTRODUCTION

We have recently showed that the spatial arrangement and temporal development of the turkey blastoderm from first cleavage division through hypoblast formation differs from that of the chicken blastoderm (Gupta and Bakst, 1993). In the course of examining the blastoderms of fresh laid turkey eggs, it became apparent that there were variations in the morphological appearance of the Stage VII blastoderm (the stage of embryonic development observed in the fresh laid turkey egg) as well as in the unfertilized germinal disc's (UGD) of fresh eggs and in eggs stored 5 d or less. Such morphological variations were observed frequently enough to warrant a more detailed investigation.

Therefore, the objective of the present study was to identify and categorize the different morphological appearances of the turkey blastoderm and UGD. Frequency estimates of each morphological category were determined within groups of hens that had been inseminated twice with either fresh or stored (24 h) semen with no additional inseminations. It was assumed that the distribution of the morphological categories between and within treatments may reflect the contribution of the "aged" sperm to blastoderm and UGD morphology. Another treatment group was inseminated weekly, and another group served as an uninseminated control. Using such treatments, differences in the frequency estimates of each morphological category due to the presence or absence of sperm would be observed if such a sperm aging phenomenon existed.

MATERIALS AND METHODS

Management of the turkey breeder flocks and artificial insemination procedures have been described elsewhere (Bakst and Cecil, 1992). Three groups of 20 hens were inseminated with a total of 200 million sperm on Days 14 and 15 after the onset of photostimulation, which began at 26 and 28 wk of age for the toms and hens, respectively. One group of hens (Stored 1X) was inseminated with semen stored at 4 C for 24 h (for details on the storage procedure see, Bakst and Cecil, 1992). The second and third groups were inseminated with fresh semen (used within 1 h of collection) either

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Abbreviation Key: UGD = unfertilized germinal disc.

only on Days 14 and 15 (Fresh 1X), or inseminated initially on Days 14 and 15 and weekly thereafter (Fresh XX). The fourth group consisted of uninseminated hens (Control).

For the first 10 wk of production, eggs were collected four or more times daily and evaluated immediately or placed in a 15 C room and examined within 5 d of collection. Eggs were opened and the appearance of the blastoderms or UGD noted with the unaided eye. If the morphology could not be readily categorized, a magnifying glass or eyepiece coupled with a narrow, intense light beam directed at the blastoderm or UGD were used in the classifying process. Data are presented in 2-wk production intervals with Period 2 representing Weeks 1 and 2 of egg production, Period 4 representing Weeks 3 and 4 of egg production, Period 6 representing Weeks 5 and 6 of egg production, Period 8 representing Weeks 7 and 8 of egg production, and Period 10 representing Weeks 9 and 10 of egg production.

A statistical analysis of the distribution of the morphological classifications of the blastoderms and UGD within and between each treatment (Stored 1X, Fresh 1X, Fresh XX, and Control) was attempted using Fisher's exact test[2] and correspondence analysis using the SAS® procedure CORRESP (SAS Institute, 1990).

RESULTS

Morphological Categories

Blastoderm and UGD classifications are found in Table 1 and are self-explanatory. Morphological differences between categories are subtle and all could not be reproduced satisfactorily using available methods of image reproduction. For example, the outermost translucent ring, the periblast (Romanoff, 1960) is clearly visible in Type A and B (Figure 1) blastoderms and not clearly visible in Type C (Figure 2) blastoderms. Similarly, the Type L UGD (Figure 3) is characterized by a dense irregular shaped white spot surrounded by vacuoles. In contrast, the Type N UGD (Figure 4) has no vacuoles surrounding the irregular shaped dense white spot.

Statistical Analyses

The size and sparseness (many low frequencies and zero observations, see Tables 2 and 3) caused difficulties for both analyses. The exact chi-square statistic could not be calculated for many of the large contingency tables and the sparseness of the data degraded the validity of the correspondence analysis.

Uninseminated Hens

With the exception of Type N, the vast majority of 424 UGD examined were characterized by the presence of vacuoles. The frequency distribution, and size of the vacuoles varied, as indicated in the defined categories (Table 1). The next most outstanding characteristic defining the UGD was the dense, small, usually stellate, centrally located white spot. The stellate appearance of the white spot reflected the density distribution of the vacuoles. The irregular shaped white spots appeared to be formed as a consequence of vacuoles abutting directly on their periphery. This can be most clearly discerned by stereomicroscopy.

Ten eggs (4.2%) possessed UGD that were classified in one of the "fertile" categories and were considered parthenogenic embryos. Six parthenotes, which were classified in the A and B blastoderm categories (Table 2), were found in Periods 4 and 6. One parthenote, which was classified in the G category, and three parthenotes, which were categorized in the F category, were observed in Periods 4 and 10, respectively. It appears that the period of egg production had little influence on the frequency and category of parthenogenic UGD.

Of all eggs categorized in the virgin hen group (Table 2), 55% (233 out of 424 eggs) and 27% (116 out of 424 eggs) were in the L and M categories, respectively. The other categories collectively accounted for about 15% of the eggs. Although the percentage of UGD in Type L ranged between 48 and 60% within each egg production period, Type M exceeded 32% only in the last three periods, and Type N exceeded 11% only in the first two periods. Within the remaining categories, only minor variation was observed between periods.

The percentage distribution of each morphological category was determined within the blastoderm and UGD categories (Table 3). Type L was clearly the most prevalent category, comprising 50 to 60% of the UGD classified. There appeared to be a tendency for Types M and O to increase and Type N to decrease in frequency with continued egg production.

Fresh XX

In this group, it is assumed that the majority of fertile eggs were fertilized by sperm residing in the oviduct for no longer than 1 wk (the interval between successive inseminations). A total 335 eggs were examined. As seen in Table 2, the percentage of true fertility was 100, 92, 84, 77, and 92%, over Periods 2, 4, 6, 8, and 10, respectively (Table 2). In each period, the A category (about 29% of all eggs laid in this treatment) of blastoderms was clearly the highest percentage, with the D category the second most frequently observed blastoderms (about 15% of all eggs laid in this treatment). The low fertility in Periods 6 and 8 remain unexplained. Infertile eggs were categorized most frequently in Type M (about 8% of all eggs laid in this treatment).

When the percentage distribution of each morphological category was determined within the fertile group (Table 3). Type A decreased (27%) over production, as did H and J but to a lesser extent. The differences between any production periods within individual Types B through K (Figures 1, 2) was equal to or less than 11%.

[2]StatXact-Turbo, Cytel Software Corp., Cambridge, MA 02138.

TABLE 1. Morphological classifications of the blastoderm and unfertilized germinal disc. Unless otherwise stated descriptions are based on observations with the unaided eye

Blastoderm	
A	Three distinct areas are observed. Near the center is a small, diffuse, whitish spot (the area alba). This is surrounded by a translucent area (area pellucida), in turn surrounded by a pale whitish ring (area opaca). Less evident and forming the outermost component of the blastoderm is the periblastic region.
B	Very similar to A. However, subtle variations in relative density of each of the three areas comprising the blastoderm. The periblast, a translucent ring surrounding the blastoderm, is clearly discernible.
C	The total blastoderm is pale and diffuse rendering the three regions (areas alba, pellucida, and opaca) difficult to discern. The area alba is just visible. This category is more frequently observed in eggs stored more than 4d.
D	Similar to C. However, the periblast lacks an outer boundary and appears to blend with the yolk.
E	Similar to A, except that the area alba is more distinct and eccentric and the area opaca is surrounded the periblast, which appears to be an incomplete ring.
F	Similar to A, except that the area alba is both a dense white color and eccentric and that the area opaca forms an incomplete ring.
G	The blastoderm has a general diffuse, pale white appearance with no clear delineation of three areas. The area alba is not visible.
H	The blastoderm has a general dense appearance due to a narrow diffuse, pale white area pellucida and the absence of the area alba.
I	Similar to A except the blastoderm is noticeably smaller.
J	The three areas are absent. A central dense white area fades peripherally and gradually appears to become continuous with the surrounding yolk. The appearance is similar to that of a less developed Stage V or VI blastoderm (Gupta and Bakst, 1993).
K	Similar to A, except that the overall diameter is larger and the area alba is absent. However, the area pellucida and area opaca are clearly visible. This blastoderm resembles a freshly laid fertile chicken egg.
Unfertilized germinal disk	
L	This morphology is the most frequently observed. It has a central, dense, small, asymmetrical, white spot which appears irregular due to numerous adjacent small vacuoles. Vacuoles are clearly observed using a stereomicroscope or a magnifying glass. A paler, whitish, symmetrical ring surrounds these structures.
M	Similar to L but some features are less distinct. Central spot may be less dense and vacuoles less conspicuous by eye, but evident by microscopy.
N	Has large, dense, somewhat symmetrical, white mass with no surrounding vacuoles but is surrounded by a clear zone.
O	Like N but has one or more large vacuoles surrounding central white mass. Smaller vacuoles may be present. Surrounded by a clear zone.
P	Three distinct zones, a dense central white area, surrounded by symmetrical clear ring and a pale, whitish ring. Similar to A blastoderm except the central white zone is more dense here.
Q	Similar to P except the outer ring is more diffuse with no outer boundary.

When the percentage distribution of each morphological category was determined within the infertile group (Table 3), Type L increased (50%) over production, as did Type O, but to a lesser extent. In Periods 4, 6, and 8 Type M was clearly the dominant UGD morphology.

Fresh 1X

The total eggs produced were 362 and the percentage of true fertility was 100, 84, 67, 27, and 4% over Periods 2, 4, 6, 8, and 10, respectively (Table 2). As hens were inseminated only prior to the onset of egg production with fresh semen, it is assumed that eggs produced in Periods 2 through 10 were fertilized by sperm "aged" *in vivo.* With respect to blastoderm morphology, Type A dominated and appeared with nearly twice the frequency of Type B over the observation periods. Type D (in Period 4) and C (in Period 5) exceeded Type A. With respect to IGD morphology, the L and M categories accounted for the 40% of all eggs produced over the production period observed.

When the percentage distribution of each morphological category was determined within the fertile group (Table 3), Type A dominated in Period 2 and 6 and Type D dominated Periods 4, 8, and 10. When the percentage distribution of each morphological category was determined within the infertile group (Table 3), Type L dominated for each period except 8, which was dominated by Type M.

Stored 1X

A total of 331 eggs were examined with fertile egg production through Period 8. As in the Fresh 1X treatment, eggs produced in Periods 2 through 8 were fertilized by sperm "aged" *in vivo.* To further exacerbate this aging effect, semen was stored *in vitro* for 24 h prior to insemination. Unlike Fresh 1X, Types A and C constituted

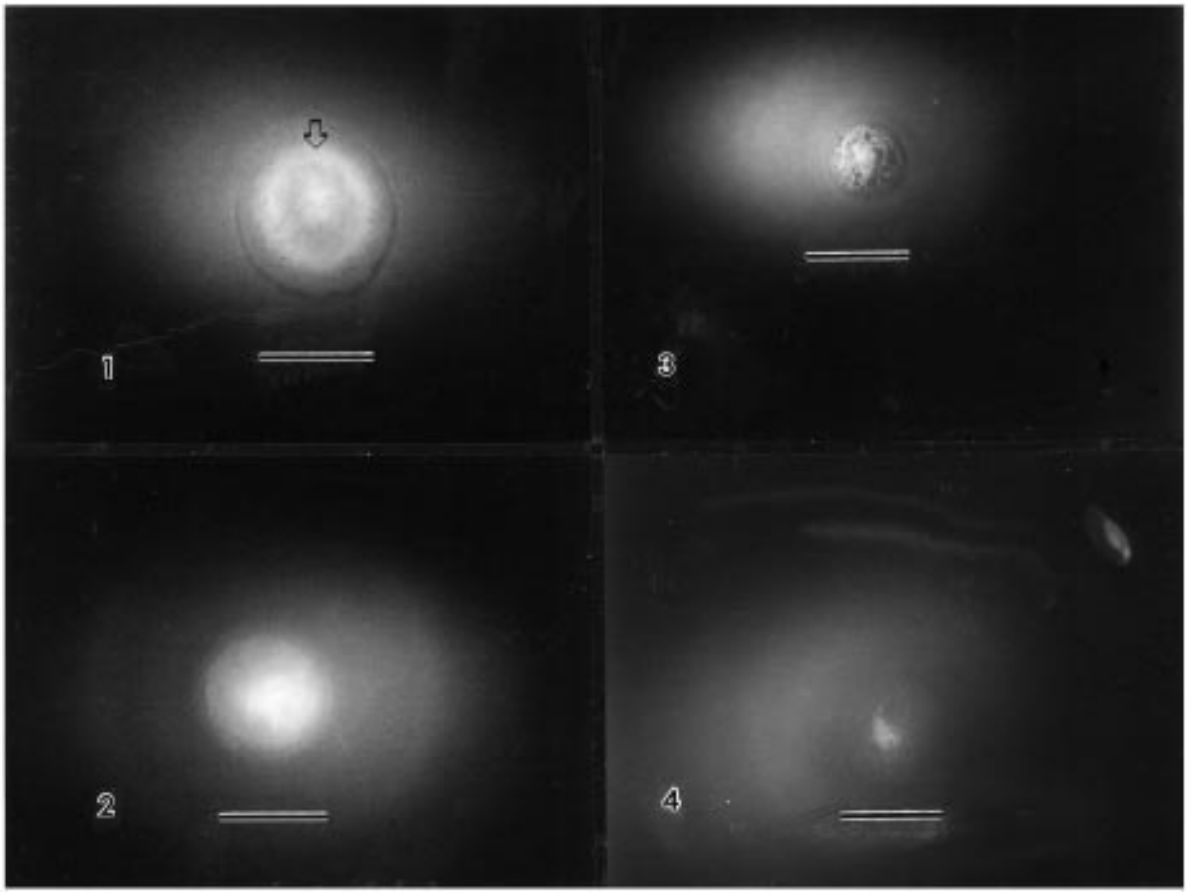

FIGURE 1. A Type B blastoderm is observed. The outermost translucent ring, the periblast, is clearly visible. The arrow highlights the area opaca, (Scale = 3.8 mm).

FIGURE 2. A Type C blastoderm is observed. Here the areas comprising the blastoderm are considerably less distinct than that seen in the Type A or B blastoderm. (Scale = 3.2 mm).

FIGURE 3. A Type L infertile germinal disc is observed. At this magnification the vacuoles surrounding the irregular shaped dense white spot are clearly visible. (Scale = 3.2 mm).

FIGURE 4. A Type N infertile germinal disc is observed. Even at this magnification, vacuoles surrounding the irregular shaped dense white spot are absent. (Scale = 3.2 mm).

25 and 15%, respectively, and Types L and M constituted 16 and 17%, respectively, of all eggs produced.

When the percentage distribution of each morphological category was determined within the fertile group (Table 3). Type A tended to decrease continuously with Periods 4, 6, and 8. Conversely, Types C and D tended to increase in the course of egg production. When the percentage distribution of each morphological category was determined within the infertile group (Table 3), Types L, M, and N dominated Periods 8, 6, and 4, respectively.

DISCUSSION

Past work describing the gross morphology of the blastoderm and UGD is quite limited. Arora and Kosin (1966) reviewed several papers published between 1872 and 1961 and briefly described the gross morphological appearance of the chicken, turkey, and duck blastoderms in unincubated eggs. They noted that most authors cited the presence of vacuoles in the UGD as an indicator of an infertile egg and the presence of vacuoles in the blastoderm as an indicator of embryonic death or eminent embryonic death. In general, observations reported herein concur with those cited above.

Without making a distinction between turkeys and chickens, Arora and Kosin (1966) described the sequence of morphological changes accompanying the storage of blastoderms at 13 C and 85% relative humidity for up to 28 d. Although noting variation among specimens, particularly in the turkey blastoderms (compare Figures 1 and 2 in Arora and Kosin, 1966), the authors failed to elaborate on such variations and restricted their classification to five primary stages and no secondary stages. Furthermore, what was referred to as the Nucleus of Pander by Arora and Kosin (1966) is actually a cluster of large cells that form the area alba unique to the turkey blastoderm (Gupta and Bakst, 1993). In contrast to the area alba, the central dense region in the UGD appears to be a result of the condensation of white yolk. Although absent in the chicken blastoderm, the area alba is a normal component of the turkey blastoderm and

TABLE 2. The distribution of blastoderm and unfertilized germinal discs (UGD) morphological categories and percent fertility of all eggs for treatment groups (Trt) Fresh (1X), Stored (1X), Fresh (XX), and Control over the production periods (Time)

			Blastoderm categories												UGD categories						
Trt	Time	N[1]	A	B	C	D	E	F	G	H	I	J	K	Total	L	M	N	O	P	Q	Total
			(%)																		
Fresh	2	43	44	23	7	5	2	5	5	9	0	0	0	100	0	0	0	0	0	0	0
(1X)	4	86	22	10	7	26	6	8	0	0	3	0	1	84	8	3	2	1	1	0	16
	6	85	16	7	21	7	4	6	2	1	1	1	0	67	20	12	0	0	1	0	33
	8	75	5	0	5	11	0	4	0	0	1	0	0	27	25	44	1	3	0	0	73
	10	73	1	0	0	3	0	0	0	0	0	0	0	4	48	30	4	11	3	0	96
Stored	2	55	38	4	24	9	7	2	2	5	0	0	7	98	0	2	0	0	0	0	2
(1X)	4	103	48	4	15	11	5	1	0	0	2	0	5	89	1	2	3	1	2	2	11
	6	91	14	0	21	8	8	5	2	1	2	1	0	63	16	19	1	1	0	0	37
	8	82	0	1	4	2	0	0	0	2	0	0	0	10	46	43	0	1	0	0	90
Fresh	2	19	47	11	0	16	5	0	0	11	5	5	0	100	0	0	0	0	0	0	0
(XX)	4	74	43	7	12	14	3	5	0	3	3	1	1	92	1	5	1	0	0	0	8
	6	77	31	3	16	10	10	3	0	5	5	0	1	84	3	13	0	0	0	0	16
	8	90	22	1	11	18	4	4	4	1	4	0	6	77	7	14	0	1	0	1	23
	10	75	19	5	7	16	9	16	8	1	7	0	4	92	4	1	1	1	0	0	8
Control	2	37	0	0	0	0	0	0	0	0	0	0	0	0	59	22	11	0	5	3	100
	4	101	2	0	0	0	0	0	1	0	0	0	0	3	60	11	13	5	8	0	97
	6	109	3	1	0	0	0	0	0	0	0	0	0	4	53	33	4	5	1	1	96
	8	77	0	0	0	0	0	0	0	0	0	0	0	0	57	32	3	5	3	0	100
	10	100	0	0	0	0	0	3	0	0	0	0	0	3	48	36	3	9	1	0	97

[1]Total number of eggs examined by treatment group within a production period.

was present in the vast majority of the blastoderms observed in this study.

The morphological differences between and within blastoderm and UGD categories defined in this study are often subtle and may neither be apparent nor discernible without prior experience in classifying such structures. Considering the latter, it is strongly recommended that before attempting true fertility determinations, which is becoming an increasingly popular procedure to monitor flock fertility (Wilson, 1995, 1997), one should first examine eggs from virgin hens and then eggs from inseminated hens of known high fertility.

TABLE 3. The percentage distribution morphological categories within blastoderm categories and within unfertilized germinal discs (UGD) categories and the number (n) of fertilized and unfertilized eggs for treatment groups (Trt) Fresh 1X, Stored 1X, Fresh XX, and Control over the production periods (Time)[1]

			Blastoderm categories												UGD categories						
Trt	Time	N[2]	A	B	C	D	E	F	G	H	I	J	K	n[3]	L	M	N	O	P	Q	n[4]
			(%)												(%)						
Fresh	2	43	44	23	7	5	2	5	5	9	0	0	0	43	0	0	0	0	0	0	0
1X	4	86	26	13	8	31	7	10	0	0	4	0	1	72	50	21	14	7	7	0	14
	6	85	25	11	32	11	5	9	4	2	2	2	0	57	61	36	0	0	4	0	28
	8	75	20	0	20	40	0	15	0	0	5	0	0	20	35	60	2	4	0	0	55
	10	73	33	0	0	67	0	0	0	0	0	0	0	3	50	31	4	11	3	0	70
Stored	2	55	39	4	24	9	7	2	2	6	0	0	7	54	0	1	0	0	0	0	1
1X	4	103	53	4	16	12	5	1	0	0	2	0	5	92	9	18	27	9	18	18	11
	6	91	23	0	33	12	12	6	4	2	4	2	0	57	44	50	3	3	0	0	34
	8	82	0	13	38	25	0	0	0	25	0	0	0	8	51	47	0	1	0	0	74
Fresh	2	19	47	11	0	16	5	0	0	11	5	5	0	19	0	0	0	0	0	0	0
1X	4	74	47	7	13	15	3	6	0	3	3	1	1	68	17	67	17	0	0	0	6
	6	77	37	3	18	12	12	3	0	6	6	0	2	65	17	83	0	0	0	0	12
	8	90	29	1	15	23	6	6	6	1	6	0	7	69	29	62	0	5	0	5	21
	10	75	20	6	7	17	10	17	9	1	7	0	4	69	50	17	17	17	0	0	6
Control	2	37	0	0	0	0	0	0	0	0	0	0	0	0	59	22	11	0	5	3	37
	4	101	67	0	0	0	0	0	33	0	0	0	0	3	62	11	13	5	8	0	98
	6	109	75	25	0	0	0	0	0	0	0	0	0	4	55	34	4	5	1	1	105
	8	77	0	0	0	0	0	0	0	0	0	0	0	0	57	32	3	5	3	0	77
	10	100	0	0	0	0	0	99	0	0	0	0	0	3	49	37	3	9	1	0	97

[1]Due to rounding-off, the sum of the percentages A through K and the sum of the percentages L through Q may not equal 100%.

[2]Total number (N) of eggs examined by treatment group within a production period.

[3]Number of blastoderms examined by treatment group within a production period.

[4]Number of blastodiscs examined by treatment group within a production period.

Only then do the subtle variations in density and appearance within as well as between the blastoderms and UGD become apparent.

The majority of the UGD are observed in Types L and M. Furthermore, when considering just the UGD from the inseminated groups, the percentage distribution of Types L and M, compared to Types N through Q, tend to increase with each successive production period (Table 2). Of significance is the effect of insemination, and possibly sperm interaction with the ovum, on the distribution of the UGD morphological categories. Type L clearly dominated in the Virgin group with 50% or more of the UGD in each period (Table 3). In contrast, in the Fresh XX and Stored 1X treatments, Type L only dominated in one period (Table 3). Possibly the different morphological appearances of the UGD in eggs laid by inseminated hens are a result of sperm-ovum interaction (not necessarily resulting in fertilization) and the subsequent effects such supernumary sperm may have on the organization of the germinal disc. Teleologically, a local activation of the ooplasm by such sperm may facilitate the events accompanying fertilization. This suggestion appears to be supported by Perry (1987), who notes that supernumary sperm may be capable of supporting normal development for a short period. Furthermore, accessory cleavage furrows noted by Gupta and Bakst (1993; see Figure 13) in the Stage II turkey embryo may be due to supernumary sperm activity.

Whether there is an association between blastoderm and UGD morphology or the age of the sperm, either resulting from storage *in vitro*, oviductal storage, or a combination of both cannot be unequivocally denied. In an intriguing article, Blike (1988) suggests that the location of the white spot within the germinal disc may indicate the basis (male or female) of infertility in the unincubated egg. She did not differentiate between the chicken and turkey egg. Assuming the hypothesis in the previous paragraph is true, it is not possible to ascertain whether the developmental failure was due to the sperm or the ovum.

Except for Type N, nearly all UGD were characterized by numerous vacuoles in their cytoplasm. For definitive identification, it is suggested that these should be viewed through a microscope, eyepiece, or magnifying glass as it is being illuminated obliquely with an intense beam of light.

The morphology of blastoderm was more variable than that of the UGD. Although the majority of the blastoderms were initially classified in the first category, as egg production continued the percentage in the first category decreased and the variability in blastoderm morphology increased. This decrease is best exemplified by examining Periods 4 and 10 in the weekly inseminated group (Fresh XX). In both periods, fertility was 92%. Although 43% of the eggs in Period 4 fell into the blastoderm Type A, only 19% of the eggs in Period 10 were of Type A (Table 2). The biological basis for this variability can only be speculative. It is assumed that with weekly inseminations, the sperm interacting with the ovum are not aged, as assumed is the case with groups Fresh 1X and Stored 1X. Therefore, the change in blastoderm morphology may be due to hen factors. These may include changes in the responses of the ovum to sperm due to the aging of the follicular oocyte (length of time in egg production) or more erratic daily ovulatory cycles resulting in the egg mass being retained in the oviduct for longer or shorter periods than normal, or may be reflective of the overall decline in egg production with increasing age of the flock. Interestingly, the appearance of the UGD does not vary significantly in Type L and increased marginally in Type M through the production periods (Tables 2 and 3).

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