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LECTIN HISTOCHEMICAL STUDIES ON CHICKEN EMBRYOS

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ABSTRACT

Studies were conducted on 164 chicken eggs with embryonic deaths at different stages of incubation *viz*,4, 6,7 15 and 21 days respectively to detect the various abnormalities occurring during their development. The study included descriptive examinations and comparisons of chicken embryos in different stages of incubation. Objective of the experiment was to study the changes if any of the selected tissues of chick embryos to lectin binding at various stages of incubation. Specimens for lectin histochemistry were fixed in 10 per cent neutral buffered formalin. Serial sections of 5µm thickness were made. Lectin histochemistry was done using Fluorescein iso-thiocyanate (FITC) conjugated lectin from *Ulex europaeus* (UEA1) and examined under Fluorescence (Leica DM 2000 LED) microscope with green filter. The lectin Concanavalin A (ConA) was also used for the study. Histochemical studies using FITC-conjugated lectin from *Ulex europaeus* revealed a general disturbance of epidermal maturation indicated by its binding to all layers of epidermis. Proventriculus exhibited decreased binding by UEA, probably owing to a changed structural and functional status. An increased response to UEA by **c**rypts of Leiberkuhn and goblet cells of duodenum might have been due to the changes in maturation as a result of degenerative changes induced. Even though there was a strong positive reaction in the islets, the reaction was weak in the exocrine part of pancreas indicating a reduced activity of the gland in the dead bird. A moderate activity in the liver also indicated the relative physiological or degenerative changes that set in after the death. A strong positive reaction to Concanavalin A by the epithelium of nasal cavity and epithelium and cartilages of larynx indicated the slow setting in of degenerative changes in the upper respiratory tract due to lesser activity of enzymes in the area. The neurons and glia of brain did not show any positive reaction to either to UEA or Concanavalin A.

Keywords: Anomalies, chicken, embryos, histochemistry, lectin

INTRODUCTION

Study of animal development provides a dynamic perspective on gross anatomy by presenting a historical view of tissue and organ relationships. Tracing complex tissue relationships back to embryonic stages often reveals a simplified pattern. Moreover, embryology being a subject of multitude of magnifications, a comprehensive study of prehatch development is essential to understand the normal variations in development and to establish a foundation for the study of various factors causing deviations from the normal development. Even though there are studies on the hatchability of chicken, the specific studies on the relationship between their degree of occurrence with the anatomical possibilities and nature of embryonic anomalies are less. With lectin histochemistry adding support to the histo-morphological observations, the present study was undertaken to elucidate the localization of lectins in the selected organs of the chicken embryos during various stages of prehatch period of development.

MATERIALS AND METHODS

This study included descriptive examination of 164 chicken eggs with embryonic deaths collected from AICRP on Poultry for eggs, Mannuthy, Revolving Fund Hatchery, Dept. of Poultry Science, College of Veterinary and Animal sciences, Mannuthy and Hatchery of Poultry Farm, CVAS, Mannuthy. The tissue samples were collected from embryos of 21st day and also from 4, 6 ,7 and 15 days of incubation from head, liver, heart, proventriculus, pancreas, skin and muscles were fixed in 10 per cent neutral buffered formalin (10% NBF), for 48 hours. The fixed specimens were washed, dehydrated and embedded in high melting paraffin (MP 58-60 ^{o}C). Serial sections of 5µm thickness were made. For lectin histochemistry, the procedure reported by West *et al.* (2012) was followed. Trypsin from bovine pancreas, Fluorescein iso-thiocyanate (FITC)-conjugated lectin from *Ulex europaeus* (UEA) and Concanavalin A procured from Sigma-Aldrich were used.

The FITC conjugated lectin was dissolved in Phosphate buffered saline (PBS, pH 7.2, 15M*)* and 0.1 per cent solution of Trypsin was prepared. Formalin fixed tissue sections of five µm thickness were deparaffinised and hydrated to distilled water. Washed in phosphate buffered saline (PBS, pH 7.2, 15M*)* for 10 minutes; treated with 0.1 per cent solution of trypsin in PBS for 30 minutes; washed in PBS for 10 minutes; covered the sections with 200 µg per litre of lectin in PBS and were kept in a moist environment for 30 minutes;

washed in PBS for 20 minutes; sections were mounted with a medium containing 90 per cent glycerol and 10 per cent PBS; and examined. Digital images were stored in under Fluorescence (Leica DM 2000 LED) microscope with green filter.

For Concanavalin A, serial dilutions of 20 µl (1mg/ml) in 180 µl PBS were used. Incubated the slides for 30mts at 56°C for deparaffinisation; followed by Xylene -10mts x 2; Absolute alcohol 10mts x 2; Methanol- H_2O_2 -20mts; Distilled water -5mts; PBS- 10mtsx3; Lectin -30mts; PBS- 10mtsx3; DAB- 20mts (last 2 mts 0.5% H_2O_2); Distilled water -10mts; counterstained with Haematoxylin; washed in tap water and dehydrated and mounted in DPX. Digital images were stored in under Fluorescence (Leica DM 2000 LED) microscope.

RESULTS AND DISCUSSION

The different hatcheries of the University farm showed good hatchability percentage due to the well maintained conditions except for the experimental groups which exhibited a lower hatchability percentage. Of the unhatched eggs, a major contribution towards lesser hatchability accounted for infertile eggs, followed by mortality during early period, then by late stages and then mid-stage mortality. Lyons (2003) also revealed that approximately 20 percent of chicken eggs normally did not hatch; the majority of this expected percentage of embryonic mortality occurred during the first and last weeks of incubation. Various factors like genetic, infectious, nutritional and managemental may be attributed to cause embryo mortality in chicken (Lalithakunjamma and Nair, 1990). Of the unhatched eggs studied, 5.52 per cent chicks were anomalous and some were weaklings.

Skin

Since the pattern of lectin binding to routinely processed sections of normal skin is related to cellular maturation, all adult epidermal cells bind UEA to the upper layers **(**Rhodes and Milton, 1998**)**. Whereas in the present study, skin showed binding of Fluorescein isothiocyanate (FITC)-conjugated lectin from *Ulex europaeus* (UEA) to all layers of epidermis, implying a general disturbance of epidermal maturation in the developing cell layers of the chick embryos (Fig. 1).

Proventriculus

Lectin binding to the normal gastric mucosa and the expression of carbohydrates in the surface mucous cells is partly dependent on secretory status. In the present study the proventriculus of the chick embryos at all stages of incubation exhibited decreased binding by UEA

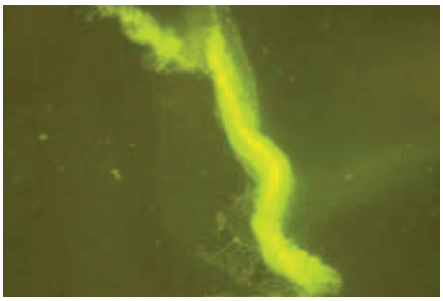

Fig. 1. Skin of chick embryo at 21 days of incubation exhibiting positive response of green fluorescence to FITC-conjugated lectin from *Ulex europaeus* (UEA) x 200

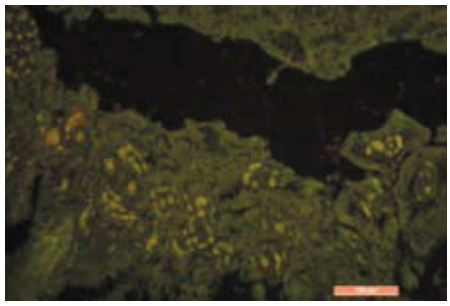

Fig. 2. Glands and mucosa of proventriculus of chick embryo at 21 days of incubation exhibiting mild response of green fluorescence to UEA x 200

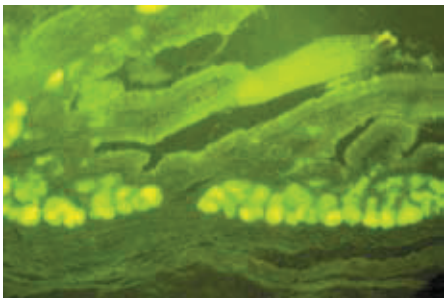

Fig. 3. Crypts of Leiberkuhn and goblet cells of duodenum in chick embryo at 21 days of incubation exhibiting positive response of green fluorescence toUEAx 200

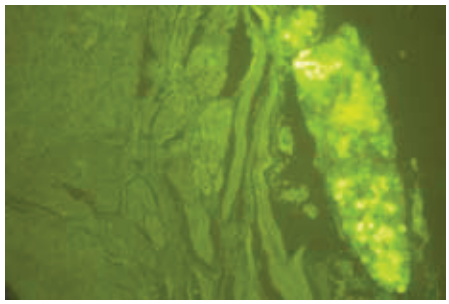

Fig. 4. Pancreatic islets of chick embryo at 21 days of incubation exhibiting positive response of green fluorescence to UEA x 200

Fig. 5. Liver of chick embryo at 21 days of incubation exhibiting moderate fluorescence to UEA x 200

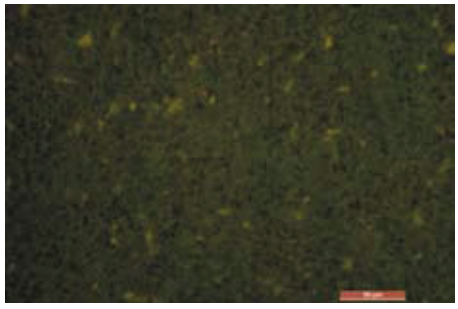

Fig. 6. Liver of chick embryo at 21 days of incubation exhibiting moderate fluorescence to UEA x 400

(Fig. 2), probably owing to increased oligosaccharide sialylation or to loss of glycosyltransferases in the dead cells due to a changed structural and functional status **(**Rhodes and Milton, 1998).

Duodenum

The present study indicated an increased response to UEA by Crypts of Leiberkuhn and goblet cells of duodenum (Fig. 3). According to Rhodes and Milton

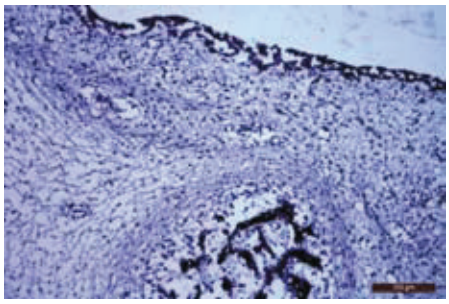

Fig. 7. Developing nasal epithelium of chick embryo at 6 days of incubation exhibiting positive reaction on lining epithelium to the lectin Concanavalin A (Con A) x 100

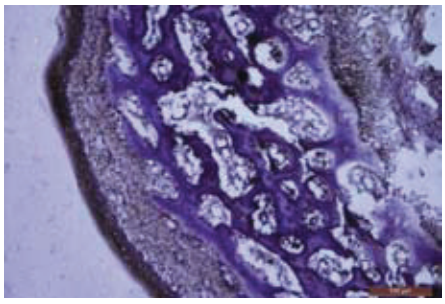

Fig. 8. Developing larynx of chick embryo at 15 days of incubation exhibiting positive reaction on lining epithelium as brown colour to Concanavalin A x 200

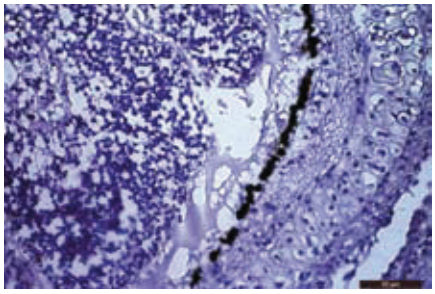

Fig. 9. Developing brain, meninges and cranium of chick embryo at 7 days of incubation exhibiting negative reaction to the Concanavalin A x 400

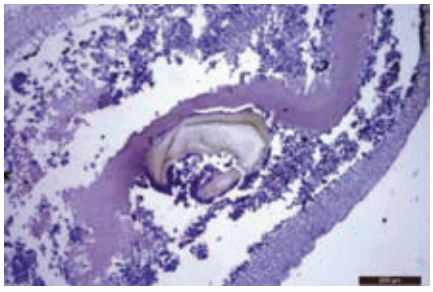

Fig. 10. Developing optic cup of chick embryo at 4 days of incubation exhibiting moderate reaction on lining epithelium to the Concanavalin A x 100

(1998), in the small intestine, glycosylation status and lectin binding change according to the site of the cell in the crypt or villus. They opined that the changes in maturation seen in diseases are reflected by increased binding by UEA, confirming the results in this study.

Pancreas

In the present study, the pancreas exhibited strong positive reaction in the islets, but the exocrine part revealed only a weak reaction (Fig. 4)**.** According to Rhodes and Milton (1998), in routinely processed tissues, UEA binds to acinar and ductal tissue. A reduced reaction by the exocrine part in the present study may indicate a reduced activity of the gland in a dead embryos.

Liver

According to Rhodes and Milton (1998), the specialized endothelial cells lining the liver sinusoids show a marked increase in UEA binding in diseases like chronic hepatitis and cirrhosis. The present study revealed only a moderate activity in the liver (Figs. 5, 6)**,** indicating the

moderate degenerative changes that set in after the death of the chick embryos.

Upper Respiratory Tract

As reported by Rhodes and Milton (1998), in the nasopharyngeal epithelium, all layers of cells bind ConA normally. To the fibrous tissue, lectins show strong binding to glucose and galactose residues linked to hydroxyproline in collagen, and this may be used to demonstrate tissue architecture and changes in collagen. Lectin histochemistry reveals changes in the function of chondrocytes in normal and degenerating cartilage, with the breakdown of bonds in fibrillated cartilage making sugars accessible. The present study also exhibited strong positive reaction to the epithelium of nasal cavity (Fig. 7) and epithelium and cartilages of larynx (Fig. 8)**,** indicating the slow setting-in of degenerative changes in the upper respiratory tract due to lesser activity of enzymes in the area.

Brain and associated structures

The brain neurons and glia did not show any positive reaction to either to UEA or Concanavalin A (Fig. 9), confirming the findings of Rhodes and Milton (1998) that in the central nervous system glia other than microglia and neurons are negative for lectins. The developing optic cup showed a moderate reaction on the developing choroid owing to the positive reaction to the vascular layer (Fig. 10).

SUMMARY

As the embryonic pattern foreshadows the structure of the different organs in the adults, to achieve an insight into the architecture of the adult system, it is essential to know how the organ system develops during embryonic life. Studies were conducted on 164 failed to hatch chicken eggs with embryonic deaths to detect the various abnormalities occurring during their development. Histochemical studies using FITC-conjugated lectin from *Ulex europaeus* revealed a general disturbance of epidermal maturation indicated by its binding to all layers of epidermis. Proventriculus exhibited decreased binding by UEA, probably owing to a changed structural and functional status. An increased response to UEA by **c**rypts of Leiberkuhn and goblet cells of duodenum might have been due to the changes in maturation as a result of degenerative changes induced. Even though there was a strong positive reaction in the islets, the reaction was weak in the exocrine part of pancreas indicating a reduced activity of the gland in the dead embryos. A moderate activity in the liver also indicated the relative physiological or degenerative changes that set in after the death. A strong positive reaction to Concanavalin A by the

epithelium of nasal cavity and epithelium and cartilages of larynx indicated the slow setting in of degenerative changes in the upper respiratory tract due to lesser activity of enzymes in the area. The neurons and glia of brain did not show any positive reaction to either to UEA or Concanavalin A**.** The diagnostic use of lectin histochemistry is still restricted to the identification of abnormal storage products in cells, of normal and neoplastic endothelial cells, and of fungi. The present study also revealed that lectins are invaluable for the study of cell-surface interactions and that the lectin-binding patterns are correlated with morphological state of the cells, whether living, dead or neoplastic.

ACKNOWLEDGEMENT

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