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The distribution of alkaline phosphatase and carbohydrates in early turkey (*Meleagris gallopavo*) embryo

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Abstract

In developmental studies of the embryo, it is very important to find suitable methods for the characterization of various tissue. This study to determine suitable markers in the development of the nervous system and other organs in turkey embryo to be a clue for exploring the role of alkaline phosphatase in the development of an embryo,perception of organ function after birth, and studies of embryogenesis of the nervous system.. The embryos from stages 19 to 31 Hamburger & Hamilton, were studied by histochemical and immunostaining techniques. The results showed that the stage-specific embryonic antigen-1 expression was restricted to the spinal cord, and other organs were negative. At stages, 19, 20 and 29 Hamburger & Hamilton, alkaline phosphatase reaction was either negative or weakly positive in embryonic organs. At stages 30-31 Hamburger & Hamilton, a strong alkaline phosphatase reaction was observed in the spinal cord, mesonephros, gonad, dorsal aorta, and liver sinusoids. Sections stained with periodic acid-Schiff confirmed the presence of glycogen in the heart, mesonephros, gonad, notochord, chondrocytes, and weakly in the ventral horn of the spinal cord. This study demonstrates that alkaline phosphatase reaction and the stage-specific embryonic antigen-1 expression are effective markers for developing the nervous system in turkey embryos. Also, we observed that both periodic acid-Schiff and alkaline phosphatase staining are useful methods to study other organs in turkey embryos.

Key words: Alkaline phosphatase, Histochemistry, Immunohistochemistry, Development, Turkey embryo

Introduction

From many years ago, avian embryo used as historical research model in classical embryology (Farzaneh et al. 2017). Alkaline phosphatase (ALP) is zinc metallo enzyme that first discovered in the 1920s and located on the outer layer of the cell membrane (Brichacek & Brown 2019, Low et al. 2020). Alkaline phosphatase have been found in the kidney, liver, intestine, bone, and other tissues in mammals and have different physiochemical properties (Kliegman et al. 2018, Low et al. 2020). Furthermore, the presence of this enzyme verified in different tissues of chicken and Japanese quial (Khillare et al. 2013). Phosphatase enzymes are essential for many biological functions and play important roles in the proliferation and

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differentiation of cells (Khillare et al. 2013, Stefkova et al. 2015, Banaee et al. 2019, Banaee 2020). Antibodies specific horseradish can be used to identify sensory cells in the neural tissue and have been able to show the mutations that are effective in the organization of the central nervous system. Stage-specific antigen-1 (SSEA-1), is a marker associated with cell to cell interaction during embryogenesis, differentiation, and neurodevelopmental process in the embryo (Koso et al. 2004, Kudo et al. 2004, Wade et al. 2014). Also, SSEA-1 is expressed on neuroepithelial cells and play important roles in cell growth (Itokazu & Uu 2015). Carbohydrates play an important role in the identification of cells and in cell- to- cell interaction (Kudo et al. 2004). After birth, there is glycogen in the liver, skeletal muscle, brain, heart, kidney (Ellingwood & cheng 2018). Glycogen function is not clear but can used as a source of energy in these organs (Adeva-Andany et al. 2016, Szymanska et al. 2021). The use of histochemical methods such as periodic acid-Schiff (PAS) and ALP staining can be clues to explore the role and biological significance of this enzyme in different stages of embryonic development and perception of organ function after birth. Also, the use of immunostaining can help to identification of neural stem cells to study of embryogenesis of the nervous system (Itokazu & Yu 2015). Turkey is an important commercial species but current embryology research performed mostly in the chick. There are few studies on turkey embryos. We demonstrate methods for studying the development of the nervous system of turkey. Finally, we introduce procedures that are useful for the evaluation other organs in turkey embryos.

Material and Methods

Embryo collection and manipulation

30 Fertile Bronze turkey (*Meleagris gallopavo*) eggs (each stage 6 eggs) were obtained from a commercial farm. Eggs were incubated at 38°C and 65% humidity with rocking every 60 minutes. Eggs were opened at the blunt end by a small scissor. The embryos were observed under a stereomicroscope and were staged according to Hamburger & Hamilton (H&H) stages (1951). Mun & Kosin (1960) have staged the embryonic development of the broad breasted Bronze turkey embryo and have reported that the H&H staging system is useful to estimate the extent of development of the turkey embryo between stages 2-36. According to the morphological changes in this staging system, we used embryos at stages 19, 20, 29, 30, and 31 (corresponding to days 4, 4.5, 8.5, 9, and 9.5 of the embryonic development in turkey, respectively). The embryos at stages 19-31 (H&H) were removed from the yolk and were fixed immediately in 4% paraformaldehyde (Sigma; USA) for 24 h at 4°C. Some specimens were fixed in gender fixative for 8 h at 4°C. Then the embryos were washed with 80% ethanol. The embryos were processed by routine tissue processing method and whole embedded in paraffin blocks (Merck; Germany). 5µm thick paraffin sections were prepared using a rotatory microtome (Leica RM 2145; Germany). These slides were kept in phosphate-buffered saline (PBS) at 4°C. Tissue sections were stained using specific methods such as PAS staining, ALP staining, monoclonal antibody, and Best's Carmine method (in the eye sample).

Alkaline phosphatase staining

Paraffin was removed from tissue sections by xylene and rehydrated in series of graded alcohol. The slides were stained for ALP activity by using NBT BCIP (Nitro-blue tetrazolium chloride 5- Bromo-4- choloro- 3´- indolyl phosphate P-Toluidine salt (BCIP) assay (Thermo Scientific; USA). As negative controls, ALP-stained sections were exposed to heat (60°C for 1 hr). For positive control, a liver tissue sample of 1- day old chick was used.

Immunohistochemistry with SSEA-1 antibody

Tissue sections were deparaffinized and rehydrated in ethanol series. To block endogenous peroxidase activity, the slides were incubated with 0.3% H_2O_2 in PBS for 15 minutes. The slides were washed and then blocked in 10% bovine serum albumin (BSA)/ PBS for 60 min to eliminate non-specific staining. Tissue sections were incubated with the primary antibody, SSEA-1 (R & D; USA) at 4°C overnight in a humidified chamber. Tissue sections were washed three times in PBS and re-incubated with the secondary antibody, donkey anti-mouse IgM conjugated to horseradish peroxidase (HRP) (Jackson Immunoresearch; USA) for one hour at room temperature. After washing in PBS, sections were incubated with Diamino benzidine (DAB) for 5 minutes. Slides were counterstained with Hematoxylin for 15 seconds. Finally, sections were dehydrated, cleared, and mounted with Entellan (Merck; Germany). As a negative control, slides were only stained with the secondary antibody.

Results

Stages 19- 20 (H&H)

The ALP reaction was negative in the neural tube at stage 19 H&H (Fig. 1, A-I). This tissue showed weakly or no positive reaction with PAS staining (Fig. 2, A-I). In addition, SSEA-1 staining was observed along the border of the neural tube (Fig. 3, A-I). The notochord located at the developing midline, ventral to the neural tube stained positive with PAS (Fig. 2, A-I) but negative with SSEA-1 antibody (Fig. 3, A-I). In this stage, PAS reaction was weakly positive in the mesonephros (Fig. 2, C-I), SSEA-1 labeled (Fig. 3, B-I) and ALP-positive cells were not found in this organ (Fig. 1, B-1). The muscular walls of the ventricle were completely free of ALP staining and this enzyme could not be detected in the endothelium of the heart (Fig. 1, C-I). Also, the heart exhibited a strong reaction with PAS staining (Fig. 2, B-1). The yolk sac endoderm was SSEA-1 and PAS-positive (Fig. 2, D-I and Fig. 3, C-I).

Stage 29 (H&H)

On the 8.5th day of incubation, the ALP reaction was negative in the spinal cord and ganglia (Fig. 1, A-II). SSEA-1 was detected in the ventricular zone, mantle zone, and white matter in the dorsal region of the spinal cord (Fig. 3, A-II). Also, the developing ventral horn and the floor plate showed a weakly positive reaction with PAS staining (Fig. 2, A-II). At this stage, no enzyme activity was observed in the notochord (Fig. 1, A-II). The notochord stained positive with PAS (Fig. 2, A-II). The ALP activity and PAS reaction were weakly positive in hepatic sinusoids but hepatocytes showed only a trace of activity (Fig. 1, C-II and Fig. 2, D-II). During the same period, SSEA-1 labeled cells were not found in the liver of turkey embryo (Fig. 3, B-II). ALP activity was weakly positive or negative in the cytoplasm of the mesonephric duct, and tubules (Fig. 1, C-II), developing gut (Fig. 1, B-II), intestine, (Fig. 1, C-II) and gonad (Fig. 1, D-II). The SSEA-1 was not detected on the mesonephric duct, tubule, and gonad (Fig. 3, C-II and D-II). Some of the PAS-positive cells were present in the gonad and heart of the turkey embryo (Fig. 2, C-II and B-II).

Stages 30- 31 (H&H)

The ALP activity was detected in the basal plate, marginal zone, and ventricular zone in the ventral region of the spinal cord (Fig. 1, A-III). The floor plate, protochondrium and notochord were negative (Fig. 1, A-III). A strong ALP activity was observed in ganglion and dorsal regions of protochondrium (Fig. 1, A-III). SSEA-1 was detected in the white matter in the dorsal region of the spinal cord (Fig. 3, A-III) but the floor plate, protochondrium and notochord were SSEA-1 negative (Fig. 3, B-III). The roof plate, notochord, and

protochondrium exhibited strong staining with PAS but the white matter did not show a positive reaction (Fig. 2, A-III). Sinusoidal capillaries in the liver stained positive using both PAS (Fig. 2, D-III) and ALP staining (Fig. 1, C-III). Although, only a trace of ALP activity and PAS- positive reaction was observed in hepatic cells of the liver (Fig. 1, C-III and Fig. 2, D-III). The ALP- positive reaction was observed in the cytoplasm of the mesonephric duct and tubule (Fig.1, B-III). At these stages, glomeruli showed little or no phosphatase, while ALP reaction was positive in bowman's capsules (Fig. 1, B-III). SSEA-1 was not detected in the mesonephric duct, tubule, and glomeruli, (Fig. 3, C-III) while these cells exhibited strong staining with PAS (Fig. 2, B-III). The ALP-positive reaction was observed in the cortex and inner medullary region of the primary gonad (Fig. 1, D-III) but SSEA-1 was not detected in this organ (Fig. 3, D-III). At these stages, PAS and ALP positive reactions were observed in the endothelium of the dorsal aorta (Fig. 2F and Fig. 1, D-III).

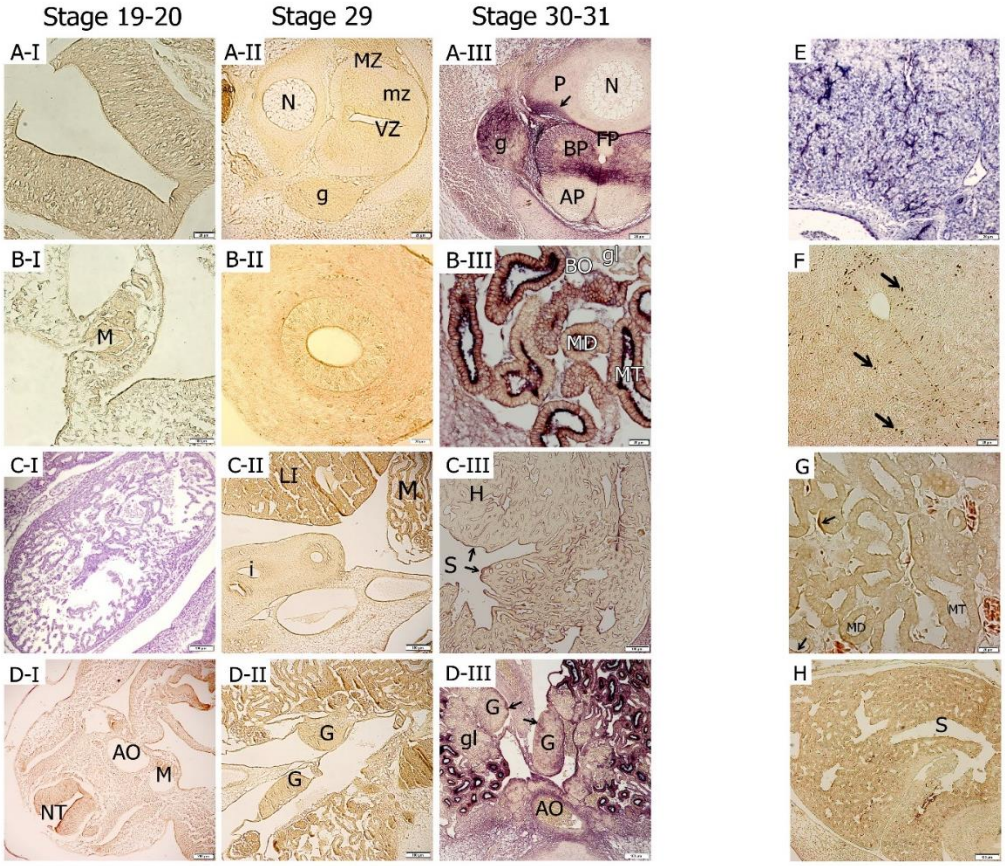

Figure 1: Alkaline phosphatase reaction in turkey embryonic tissues. At stages 19-20 H&H, the ALP reaction is negative in the neural tube (A-I), mesonephros (B-I), heart (C-I), and the cross-section of a day-4.5 turkey embryo (D-I). The ALP reaction is negative in the ventricular zone (VZ), mantle zone (mz), marginal zone (MZ), and notochord and ganglion (A-II) (stage29 H&H). The enzyme activity is negative in the gut (B-II), intestine (C-II), and gonad (D-II). The ALP reaction is weakly positive in the mesonephros and hepatocytes of the liver (C-II). At stages 30-31 H&H, strong ALP positive reaction observed in the basal plate, ganglion and the dorsal region of protochondrium (P) (arrow), but it is negative in the floor plate and notochord (A-III). ALP positive reaction was observed in the sinusoidal capillaries in the liver (C-III) (arrows), mesonephric duct and tubule (B-III), gonad (D-III), dorsal aorta (AO) (D-III), and in bowman's capsules (B-III), but the reaction was negative in glomeruli (gl) (B-III, D-III). The positive control for ALP activity (E). The residual ALP activity after heat-inactivation is shown in the spinal cord (F) (arrows), mesonephros (G) (arrows) and the liver (H). Gonad: G, Intestine: i, Bowman's capsules: BO, Mesonephros: M, Liver: LI, Notochord: N, Ganglion: g, Sinusoidal Capillaries: S, Mesonephric Duct: MD, Mesonephric Tubule: MT, Basal Plate: BP, Floor Plate: FP, Neural tube: NT.

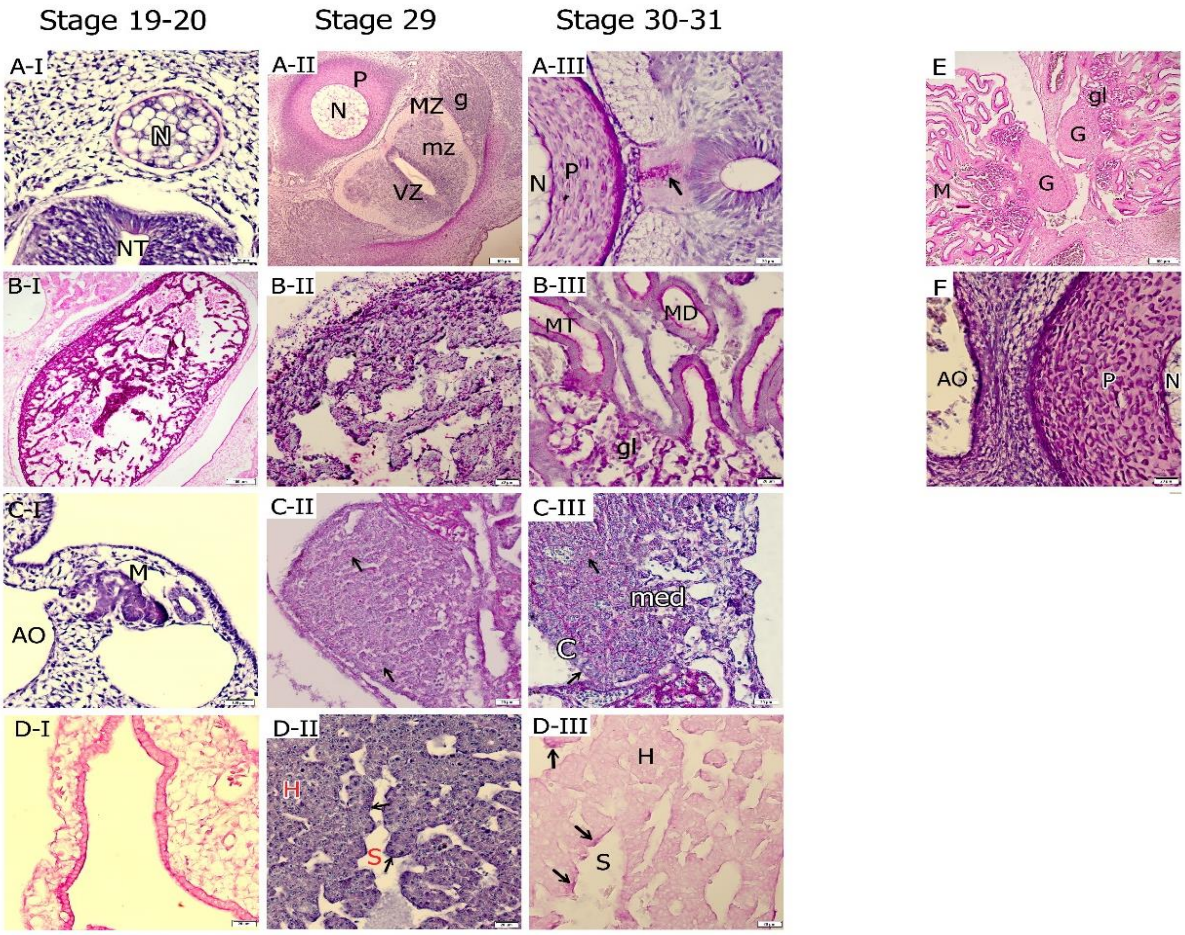

Figure 2: Periodic acid-Schiff staining in the tissues of turkey embryo. At stages 19-20 H&H, PAS reaction is negative in the neural tube (A-I), ventricular zone (VZ), mantle zone (mz), marginal zone (MZ) and the ganglion (g) of the stages 29-31 H&H (A-II). The notochord (A-I, A-II, A-III, F), chondrocytes of protochondrium (A-II, A-III, F) and the floor plate of the spinal cord (A-III) (arrow) stained positive with PAS. A strong PAS positive reaction was observed in the heart at stages 20 H&H (B-I) and 29 H&H (B-II). The PAS reaction is weakly positive in the mesonephros of the stage 20 H&H (C-I), and is strongly positive in the mesonephric duct, tubule and glomeruli (stages 30-31H&H) (B-III). Note PAS positive germ cells in the cortex and medulla of turkey gonad (arrows) (C-II, C-III). At stages 29-31 H&H, the hepatic sinusoids and hepatocytes stained weakly positive by PAS staining (arrows) (D-II, D-III). The PAS positive reaction was observed in the yolk sac endoderm (D-I). The primary gonad on the ventromedial surface of the mesonephros in the turkey embryo (E). Gonad: G, Mesonephros: M, Hepatic Sinusoids (S), Cortex: C, Medulla: med, Glomeruli: gl, Notochord: N, Neural Tube: NT, Mesonephric Duct: MD, Mesonephric Tubule: MT, Protochondrium: P.

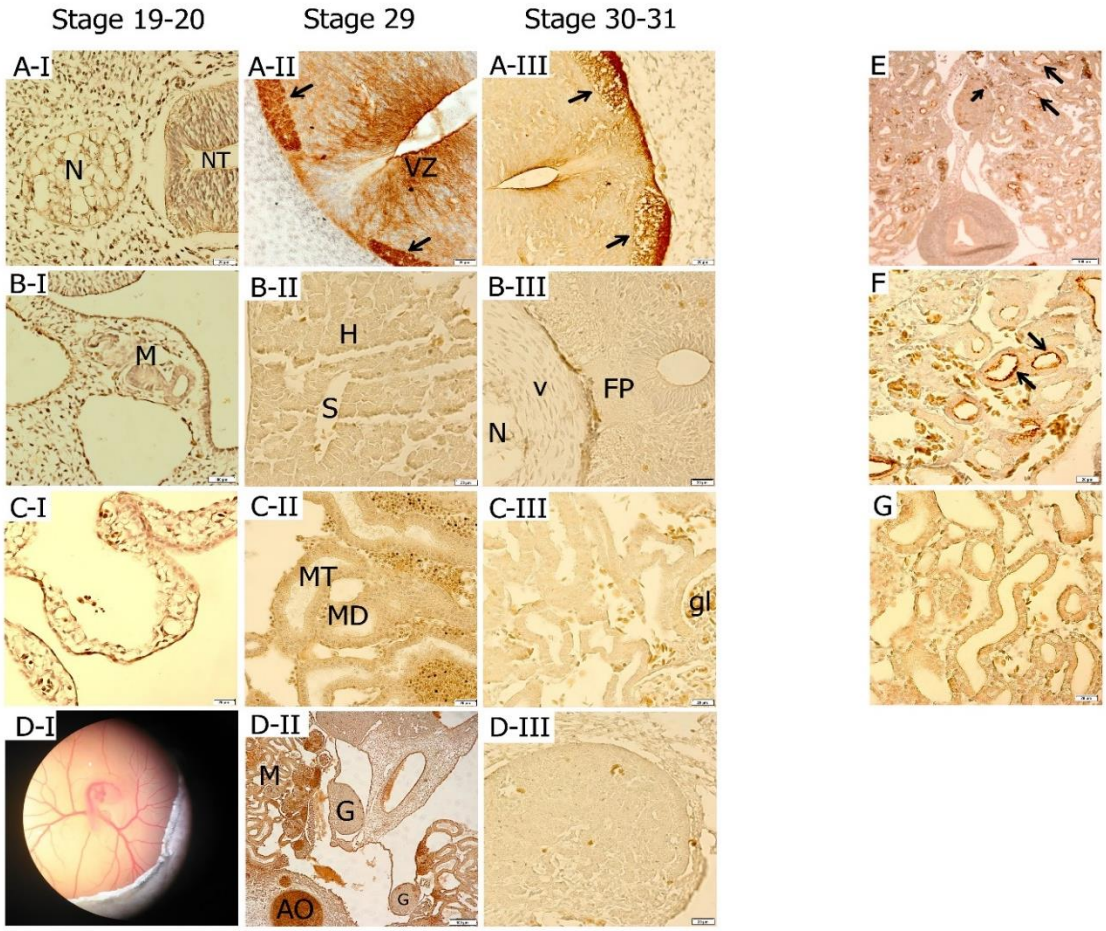

Figure 3: SSEA-1 expression in the tissues of the turkey embryo. At stages 19-20 H&H, SSEA-1 is not expressed by mesonephros (B-I) and notochord (A-I), but is expressed in the yolk sac (C-I). At stages 29-31 H&H, the SSEA-1 positive cells were observed in the ventricular zone (A-II), and white matter in the dorsal region of the spinal cord (arrows) (A-III). Note SSEA-1 negative cells in the floor plate, future vertebral centrum (V) and notochord (B-III). SSEA-1 expression was not observed in the mesonephric duct, tubule (C-II), glomeruli (C-III), hepatocytes and sinusoids of the liver (B-II) and gonad (D-II, D-III). In the positive control for immunostaining with SSEA-1, germ cells were stained in the gonad at stage 28 H&H in the chick embryo (arrow) (E) and in the mesonephros (arrows) (E, F). In the negative control, the mesonephric duct and tubule did not express SSEA-1 in the chick embryo (G). The real image (D-I) of the turkey embryo at stage 20 H&H. Mesonephros: M, Notochord: N, Glomeruli: gl, Sinusoids: S, Ventricular Zone: VZ, Floor Plate: FP, Mesonephric Duct: MD, Mesonephric Tubule: MT.

At stage 22 H&H (5.5 days of incubation), the retina contained three layers: sensory layer, pigment layer and mesenchymal cells in the outside of the optic cup (Fig. 4I). At this stage, the lens fibers did not express the SSEA-1 epitope (Fig. 4F) and these cells did not have positive PAS staining (Fig. 4C). At stage 30 (H&H), SSEA-1 was detected a long the pigment layer of the retina (Fig. 4A). The presence of glycogen was verified using both PAS (Fig. 4B) and Best's Carmine methods in the retina (Fig. 4E). Also, the retina had a positive reaction with ALP staining (Fig. 4D).

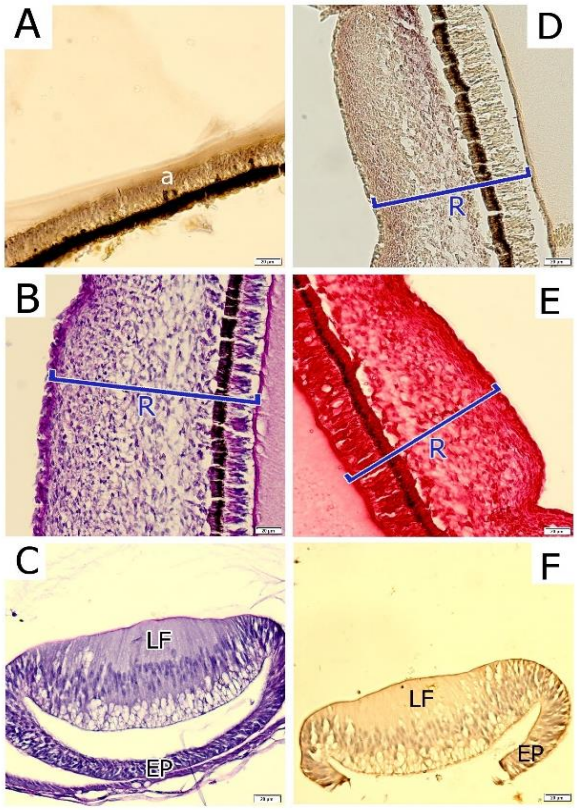

Figure 4: At stage 30 (H&H), SSEA-1 was detected along the pigment layer of the retina (A). The retina has positive reaction with ALP (D), PAS (B) and Best's Carmine (E) methods. Note the SSEA-1 (F) and PAS negative cells (C) in the lens of the turkey embryo. PL: pigment layer, SL: sensory layer, R: retina. L: lens, LF: lens fibers, EP: epithelium of lens.

Discussion

Avian embryo is a model for exploring vertebrate developmental biology (Burggren et al. 2016). In the studies of the nervous system, it is very important to find a marker for identification of embryonic stem cells in the neural tissue (Itokazu & Yu 2015). The neural tube is composed of three layers: marginal layer, mantle layer and ependymal layer (McGeady et al. 2006). In the avian embryo, at stage 22 (H&H), grey and white matter can be recognized. At stage 31 (H&H), the dorsal and ventral horns are visible in grey matter of the spinal cord. We and D'costa and Pettite (1999) have observed that, SSEA-1 is detected by neural epithelial cells in the dorsal region of the spinal cord. Also, SSEA-1 expression was confirmed in stem and neuroephithelial cells of the neural tissues (Itokazu & Yu 2015). It seems that, SSEA-1 expression in the dorsal region of the spinal cord, confirm the presence of epitopes on the sensory component of the nervous system. PAS stain is present as sensitive and specific method for diagnosis (Nabbale et al. 2014). Bastian et al. 2019, reported that decrease of level of glycogen could be essential for axon function. According to (Vukovic & Lucic 2005) glycogen-rich cells has been observed in the central canal of the spinal cord but these cells were not observed in this study. We suggested that, these cells are present only in the next stages of development in turkey embryo. The ALP is a ubiquitous enzyme that hydrolysis of phosphate esters group and found in all living organisms (Sabatakou et al. 2007, Stefkova et al. 2015, Banaee 2020). Moog (1944) applied histochemical techniques for detection of phosphatase in the chick embryo. ALP are present in different body tissue but the highest concentration of ALP is reported in the spinal cord in the chick and mammals (Moog 1944, Mori 1965, Lowe et al. 2020). It is in agreement with our finding in turkey embryo. These finding indicated that, changes in enzyme distribution and enzyme activity might play important roles in the process of histological differentiation (Moog 1944; Khillare et al. 2013, Gomes et al. 2017).

The organization of vertebrate liver, has been studied by some researchers (Abdel-Moniem et al. 2000, Doaa et al. 2013). The researchers showed that ALP is present in the canalicular membrane of the hepatocyte (Lowe et al 2020). Study in the chick, confirmed that liver cells to be completely free of ALP before glycogen be stored in this organ (Moog 1944). Raisi et al. (2018) reported that ALP activity in hepatocytes can increase the rate of glycogen in the liver. It is in agreement with the results obtained in this study. In mammals, the isoform of glycogen synthase encoded Glycogen Synthase 2 (GYS_2) gene, and glycogen function in the liver, is different of

other organs (Pederson et al. 2004, Banaee 2020). Therefore, the presence of glycogen in the liver can be related to organ function. Also, increase of ALP activity is due to the rise in serum ALP activity and can play a vital role in increase the rate of energy of cells, hepatocyte function and detoxification activity in these cells (Banaee et al. 2019, Lowe et al. 2020, Saha & Kaviraj 2009). On the other hand, it can be suggested that absence of SSEA-1 epitope in hepatocytes of the liver is not essential for embryogenesis in turkey embryo.

In birds, development of the gonad began on the 3th day of incubation (Stage 20 H&H). Then, the gonad was formed from the genital ridge at next stages of embryonic development (Peeters et al. 2009). Female gonads are formed of the primary sex cords that are contained of primordial germ cells (PGCs), somatic cells and mesenchymal cells (Kheirabadi et al. 2014). Finally, sexual differentiation of the gonads is occurred on the 5th days of incubation (Chang et al. 2012). ALP activity has been reported in germ cells that were isolated from chick embryo gonad (Naeemipour & Bassami 2013). Germ cells were showed highest ALP activity in mammal and bird (Hahnel et al. 1990, Soto-Suazo & Zorn, 2005). Also, presence of glycogen in PGCs verified using PAS staining (Wade et al. 2014). We suggested that, colonization of the gonads by PGCs could be effective in positive reaction with ALP and PAS staining's. Also, with due attention to the role of PGCs in the formation of the gamete, the presence of ALP in these cells is a reason for the role of this enzyme in the development of embryo and the function of this organ after birth. According to some researches, SSEA-1 positive germ cells could be identified in vitro studies (Kudo et al. 2004, Jung et al. 2005). As previously reported (D'costa & Petitte 1999) and in the present study, PGCs could not be detected in the turkey gonad. We suggested that this lack of expression of this epitope, take place once turkey PGCs colonized in the gonad with unclear reasons.

A morphological study was showed that the mesonephros as a transient kidney, was appeared at stage 20 (H&H) and was replaced by metanephros at stage 41 (H&H) (Gabrielli & Accili 2010, Bolin & Burggren 2013). The previous study by (Klusonová & Zemanova 2007) have been showed morphological development of avian kidney. Glycogen and ALP has been identified in the kidney (Lowe et al. 2020, Ellingwood & Cheng 2018). These observations indicate that in the mesonephros, high concentration of ALP may be connected with absorption of glucose in kidney tubules (Tapson et al. 1998, Oh et al. 2015). On the other hand, high level of enzyme activity maybe related with differentiation and functioning of this organ (Moog 1944, Yamazoe et al. 2016). SSEA-1 expression, was observed in the tubules of the fetal rat kidney (Kudo et al. 2004). The present study has not been able to find SSEA-1 labeled cells on the mesonephros but the reason for the negative staining is unknown.

In this study, we observe the residual ALP activity after inactivation of tissue sections (the mesonephros, liver and spinal cord) with heat. ALP is divided into 12 isozymes (Banaee 2020). The results of previous studies were indicated that abundant ALP isozymes in the liver, bone and kidney, were tissue nonspecific alkaline phosphatase (TNAP) (Khailova et al. 2020). Also, these isozymes were found in the brain and spinal cord (Stefkova et al. 2015, Brichacek et al. 2019*)*. According to (Dehghani et al. 2000), there was residual activity over the heat- inactivated in the mouse with absence of embryonic alkaline phosphatase (EAP) gene. Therefore, it seems that, probably there are inactivated EAP gene and TNAP isozymes in the mesonephros, liver and spinal cord of turkey embryo but more studies on bird are needed.

The cardiovascular system is the first functional system of the body in embryonic development (Witting & Munsterberg 2016). At stage 9 (H&H), a primitive heart tube was Formed in the chick embryo. The myocardial cells were allocated during the early stages of avian embryonic development and evolution of these cells were continued after birth and adulthood (Witting & Munsterberg 2016). We observed that myocardium of the heart was free of phosphatase. Researchers reported that ALP activity in the heart is low (Khailova 2020). Also, this finding is in agreement with early studies in chick embryo (Moog 1944). It thus seems that, the enzyme plays important roles at a more advanced stages of differentiation. The presence of glycogen verified in a variety of tissues such as liver, muscle, heart, kidney and brain (Ellingwood & Cheng 2018, Migocka- Patrzalek & Elias 2021). Also, researchers reported that glycogen was presented during embryonic development (Vukovic & Lucic 2005). The role of glycogen was shown as an important fuel reserve, but no information was provided regarding glycogen function (Adeva-Andany 2016, Szymanska et al 2021, Pederson et al. 2004, Saha & Kaviraj 2009). According to previous studies, cardiac glycogen had a changeable level during embryonic development and the presence of glycogen in the cardio myocytes were essential for normal heart development. Furthermore, glycogen may have a specific role in this organ (Pederson et al. 2004).

The ALP was demonstrated in the small intestine of various animal species (Gomes et al. 2017, Lowe et al. 2020, Sabatakou et al. 2007). The enzyme activity could be detected in the microvilli of the intestinal epithelium (Mc Connell 2009). This enzyme plays important roles in regulation of the intestine PH, detoxification and absorption of lipids (Lalles 2014). Researchers reported that intestine has high ALP activity (Khailova 2020). The intestinal epithelium was free of ALP activity after 8 days of incubation in the chick embryo (Moog 1950). This finding are in agreement with our results in the turkey embryo. After hatching, the intestinal brush borders were showed considerable deposits of enzyme (Sabatakou et al. 2007). Similar finding has been reported in the small intestine of rat. They reported that, there was regulatory control during the development of small intestine in rat (Gomes et al. 2017). Thus, it can be suggested that ALP activity was varied and change with time and species. Also, a physiological role of this enzyme could be, participating in digestive activity (Sabatakou et al. 2007, Lalles 2014).

Protochondrium of future vertebral centrum was free of phosphatase in the chick embryo (Moog 1943). Our results (at stage 29 H&H) are in agreement with this study. Scientists showed relation between chondrogenesis and ALP activity (Kudo et al. 2004). Therefore, ALP-positive reaction that was observed in the next stages, can justify the role of this enzyme in the formation of hard tissue. We suggest that, absence of SSEA-1 epitope in protochondrium was not essential for chondrogenesis. The presence of glycogen was verified in the proliferating of chondrocytes and growth plate cartilage. Although, glycogen content in chondrogenic cells was changeable with differentiation of cartilage (Daimont 1977, Uchimura et al. 2017).

From the beginning of embryonic development, eye was distinguishable organ but little is known about the development of it (Mitkus et al 2018). Most studies on eye development, accomplished in the embryo of the birds (Smelser 1965, Duman et al. 2019). The retina is a thin layer of eye tissue but play very important role in capture light and send a signal to the brain (Mitkus et al 2018). At stage 15 (H&H), this layer consists of neuroepithelium and the retinal pigment epithelium in chick embryo (Chu & Grunwald 1990). Then, on the 4th days of

incubation, were observed three layer in the retina: sensory layer, pigment layer and mesenchymal layer (Chu & Grunwald 1990, McGeady et al. 2006). In this study, the retina has shown positive reaction using both histochemical and immunohistochemical techniques. The Best's Carmine is a staining method to detect of glycogen (Nabbale et al. 2014). Thus, double staining of sections with PAS and Best's Carmine stains confirmed the presence of glycogen in the retina. We suggest that, glycogen is a source of energy in the retina. The ALP activity can be used as a marker for differentiation of cells (Stefkova et al. 2015). It seems that this enzyme has certain relation to the cellular differentiation in the retina. The SSEA-1 labeled cells were detected in the retinal progenitor cells (Koso et al. 2005). We observed SSEA-1 expression in cells of pigmented layer of the retina. Therefore, it can be suggested that these antigens play important roles during development of the retina.

Carbohydrates play an important role in the identification of cells and cell- to- cell interaction. We suggest that double staining of embryonic sections using SSEA-1 antibody and ALP staining is the most optimal approach to studying the development of the nervous system in turkey embryos.

Furthermore, using both PAS and ALP staining are useful to study other organs during embryogenic processes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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توزیع آلکالین فسفاتاز و کربوهیدرات‌ها در مراحل اولیه رویانی بوقلمون

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چکیده

در مطالعات تکوینی رویان، تعیین روش‌های مناسب شناسایی بافت‌های مختلف بسیار اهمیت دارد. هدف از این مطالعه، تعیین نشان‌گر مناسب در تکوین سیستم عصبی و سایر اندام‌ها در رویان بوقلمون بوده تا راهنمایی باشد در جهت کشف نقش آنزیم آلکالین فسفاتاز در تکوین رویان، درک بهتر عملکرد اندام پس از تولد و مطالعات روند جنینی تشکیل دستگاه عصبی. رویان‌های بوقلمون در مراحل (H&H)۱۹-۳۱، با استفاده از تکنیک‌های هیستوشیمی و ایمونوهیستوشیمی مطالعه شدند. نتایج نشان داد که سلول‌ها تنها در طناب نخاعی، قادر به بیان نشان‌گر سطحی SSEA-1 بوده و سایر اندام‌های مطالعه شده از نظر بیان این مارکر، منفی بودند. اگر چه در مراحل (H&H) ۱۹-۳۱، فعالیت آلکالین فسفاتازی منفی یا بسیار ضعیف بود اما در ۹/۵-۹ روز پس از انکوباسیون، واکنش قلیایی مثبت در بخش‌هایی از طناب نخاعی، مزونفروز، گناد، آئورت پشتی و سینوزوئید کبدی مشاهده گردید. برش‌های رنگ‌آمیزی شده با تکنیک هیستوشیمی پریودیک اسید شیف (PAS) نیز حضور گلیکوژن در قلب، مزونفروز، گناد، نوتوکورد، کندروسیت و بخش‌هایی از طناب نخاعی را تأیید کرد. نتایج به دست آمده از تحقیق حاضر نشان می‌دهند که آنزیم آلکالین فسفاتاز و بیان SSEA-1 می‌توانند نشان‌گرهای مناسبی در مطالعات تکوینی سیستم عصبی رویان باشند. همچنین می‌توان از تکنیک‌های هیستوشیمی پریودیک اسید شیف و آلکالین فسفاتاز قلیایی نیز به منظور مطالعه دیگر اندام‌ها در رویان بوقلمون استفاده کرد.

کلمات کلیدی: آلکالین فسفاتاز، کربوهیدرات، ایمنوهیستوشیمی، تکوین، رویان

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