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Immunohistochemical distribution of HGF, IGF-I, FGF-2, and TGF-α in kidney and liver tissues of geese (*Anser anser*) during hatching and post-hatching periods

Dilem Gülece Ermutlu* and Şahin Aslan

Department of Histology and Embryology, Faculty of Veterinary Medicine, Kafkas University, Kars, Türkey

*Corresponding author's Email: dilemermutlu@gmail.com

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ABSTRACT

Immunohistochemical studies during embryonic development of poultry are quite limited. The present study aimed to investigate the distribution of Hepatocyte growth factor (HGF), insulin-like growth factor-I (IGF-I), fibroblast growth factor-2 (FGF-2), and transforming growth factor-α (TGF-α) in the kidney and liver tissues of hatching and post-hatching geese via histologic and immunohistochemical methods. A total of 150 fertile goose eggs were used in the study. Throughout the incubation process (29 days), four eggs were used each day, starting from the sixth day, and the embryos were examined. The embryos were fixed in 10% formaldehyde solution. Geese at the 7, 15, 30, and 210 (adult) days after hatching were euthanized for a post-hatching evaluation. Routine histological procedures were performed on the kidney and liver tissues. For histological examinations were used Crossmonn's triple staining, Periodic Acid-Schiff (PAS), and Haematoxylin and Eosin (H&E) staining. HGF, IGF-I, FGF-2, and TGF-α immunoreactivities were investigated in the histological slides. An examination of kidney tissue development indicated that metanephric blastema formation started on day 10. The mesonephros was replaced by the metanephros, the permanent kidney, starting on the day 25. An examination of liver tissue development revealed that sinusoids began to narrow, hepatic plaques formed, and endothelial and Kupffer cells became distinguished as early as day 8. Upon general evaluation, FGF-2 was found to be the growth factor with the most intense immunoreactivity in the liver and kidney, while IGF-I had the least immunoreactivity. There is a paucity of studies on growth factors during the embryonic period in poultry. In the present study, all four growth factors were immunohistochemically investigated in the kidney and liver during the hatching and post-hatching periods. Because poultry have a shorter embryonic period than mammals, they need to utilize growth factors effectively and at high levels. The present study demonstrated that HGF, IGF-I, FGF-2, and TGF-α growth factors were effective in kidney and liver development during the incubation period in geese.

Keywords: Embryo, Goose, Growth factors, Kidney, Liver

INTRODUCTION

Similar to mammals, poultry kidneys are the main excretory organs that play an important role in the process of removing waste products that can harm the body. However, they differ from the lobule structure of the mammalian kidney (Scanes and Dridi, 2021). Poultry kidneys consist of a pair of organs situated bilaterally within the os lumbosacrale (Aslan, 2018). The kidneys develop from the dorsal mesoderm in three stages, namely, pronephros, mesonephros, and metanephros (Hassa and Aştı, 1997; McGeady et al., 2006). The pronephros develops from the mesoderm and begins to differentiate on day 3 of embryonic development, where it is replaced by the mesonephros (Bolin and Burggren, 2013). Mesonephros development initiates on days 3-4, becomes functional between days 5 and 11, undergoes degeneration in chicken embryos by day 15, and is subsequently replaced by the metanephros (Neuhaus and Hollemann, 2009; Bolin and Burggren, 2013). The metanephros or permanent kidney develops from the metanephric blastema of the intermediate mesoderm (Hassa and Aştı, 1997) and initiates an active process of fluid absorption through the renal corpuscles and proximal convoluted tubules on embryonic day 12 in chickens (Bolin and Burggren, 2013).

In poultry, the liver is the largest gland in the body, located in the ventral hepatic peritoneal cavity, and consists of two or three lobes depending on the species (König et al., 2016). The liver's parenchyma has an endodermal origin, and the stroma has a mesodermal origin (McGeady et al., 2006). Following the onset of liver diverticulum formation, hematopoietic cells invade the embryonic liver, thereby establishing it as the primary hematopoietic organ (Hassa and Aştı, 1997; McGeady et al., 2006). Hematopoietic cells develop rapidly and begin to form hematoblasts and other cells of the liver (Yang et al., 2019).

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Growth factors have different effects, including angiogenesis, chemotaxis, cell proliferation, fibroblast migration, wound healing, and collagen production, and are protein substances that affect cell activity both *in vivo* and *in vitro* (Tekinarslan et al., 2011). Insulin-like growth factors are synthesized predominantly in the liver but are also produced in many tissues, including the kidney (Le Roith, 1997; Le Roith et al., 2001). Insulin-like growth factors (IGFs) play important roles in various functions, including follicle growth and subsequent embryonic development (Choi et al., 2024). Although HGF is primarily associated with the liver, in a study in rats, HGF expression was four times greater in the kidney than in the liver (Liu, 2002). Studies on HGF in rats and mice have suggested that it plays an important role in kidney development, maintenance of normal kidney structure and function, and tubular repair (Matsumoto and Nakamura, 2001; Davies, 2001; Liu, 2002). In human embryos, FGF-2 plays an important role at various stages of embryonic development, including early liver development and stimulation of kidney tubules (Carlson, 2018).

Previous studies on rats have indicated that TGF-α serves as a potent mitogen for hepatocytes and is predominantly produced by hepatocytes undergoing DNA replication (Webber et al., 1994). Furthermore, high levels of TGF-α transcripts have been observed in certain structures, including the mesonephric tubules of the developing kidney (Derynck, 1992).

The functions of growth factors during the early and late embryonic periods attract a lot of interest in developmental biology. Although there is information about the effects of growth factors on cells in cell culture studies, immunohistochemical studies are quite limited. This study aimed to examine the development of the liver and kidney according to days during hatching and post-hatching periods and to determine the expression location of growth factors (HGF, IGF-I, FGF-2, and TGF-α) in these organs.

MATERIALS AND METHODS

Ethical approval

The required approval was obtained from the Local Ethics Committee of Kafkas University Animal Experiments (KAU-HADYEK/2021-171), Kars, Turkey, before the commencement of the study.

Materials

In the present study, eggs were collected daily from the sixth day of incubation (4 goose eggs per day) until the day 29. The kidney and liver tissues of the collected embryos were examined. On the day 29 of incubation, the geese hatched. This day (day 29) was considered day 0, and the liver and kidney tissues of the geese were examined on days 7, 15, 30, and 210 (adult stage; 4 geese per day). No specific euthanasia method was applied to geese during the incubation period. Euthanasia was performed by cervical dislocation on days 0, 7, 15, and 30. One hundred fifty fertilized goose eggs were obtained from the Kafkas University Veterinary Faculty Application and Research Farm, Kars, Türkiye. The eggs were sterilized via fumigation in a formaldehyde-potassium permanganate mixture (Tilki and Saatci, 2013) and placed in an incubator for 29 days (CIMUKA DF-103, Türkiye) within the goose unit, maintaining conditions of 37.5°C, 60% humidity, and rotation of 180° every 2 hours.

Methods

Starting from day 6 of incubation, four eggs were used each day, and the embryo was removed and fixed in 10% formaldehyde. The hatched geese were raised in cages in the goose unit. Water and feed were given *ad libitum*, lighting is 18 hours of light and 6 hours of darkness daily, temperature is 27-30°C (Tilki and Saatci, 2013). The hatching day was set to day 0. The geese at days 7, 15, 30, and 210 (adults) following hatching were euthanized on the indicated days for post-hatching evaluation, and their organs were fixed in 10% formaldehyde (Figure 1).

Tissue collection and preparation phase

Starting on day 6 of incubation, four embryos were removed daily and fixed in 10% formaldehyde. The heads of the embryos were removed, and the trunks were divided whole on days 6-10, in half crosswise on days 11-15, in thirds crosswise on days 16-20. And after day 20, the livers and kidneys were removed and embedded in paraffin. Routine histological procedures; Alcohol (70%, 80%, 90%, 96%, Absolute), Xylol (I, II, III), Xylol and paraffin and paraffin embedding) were performed on the tissues, and 5 µm thick sections were excised from these tissues.

Histochemical examination

Crossmonn's triple staining, Periodic Acid-Schiff (PAS), and Hematoxylin and Eosin (H&E) staining were performed for histological examinations. The histological slides were examined and photographed under a light microscope (Olympus BX51, Japan).

Immunohistochemical examination

The avidin-biotin-peroxidase complex technique (Hsu, 1981; Bingöl et al., 2024) was used to determine the immunohistochemical localization of HGF, IGF-I, FGF-2, and TGF-α antibodies in the kidney and liver tissues. Sections were incubated with anti-HGF (Santa-Cruz, sc-57193; 1:200), anti-IGF-I (Santa-Cruz, sc-74116; 1:25), anti-FGF-2

(Santa-Cruz, sc-74412; 1:100), and anti-TGF-α (Santa-Cruz, sc-374433; 1:100) for 12-16 hours at room temperature (20-22°C). Upon incubation, the sections were washed with phosphate-buffered saline (PBS), and the secondary antibodies (Biotinylated Goat Anti-Rabbit [Lab. Vision, 510.991.2800]) were added and maintained at room temperature for 30 minutes. Streptavidin horse-radish peroxidase was added to the sections, washed in PBS (3×5 minutes), and maintained for 30 minutes. Mayer's hematoxylin was used to stain the nuclei. Only the PBS solution was applied to the negative control group tissues (Figure 2). The preparations were examined under a light microscope (Olympus Bx51, Japan).

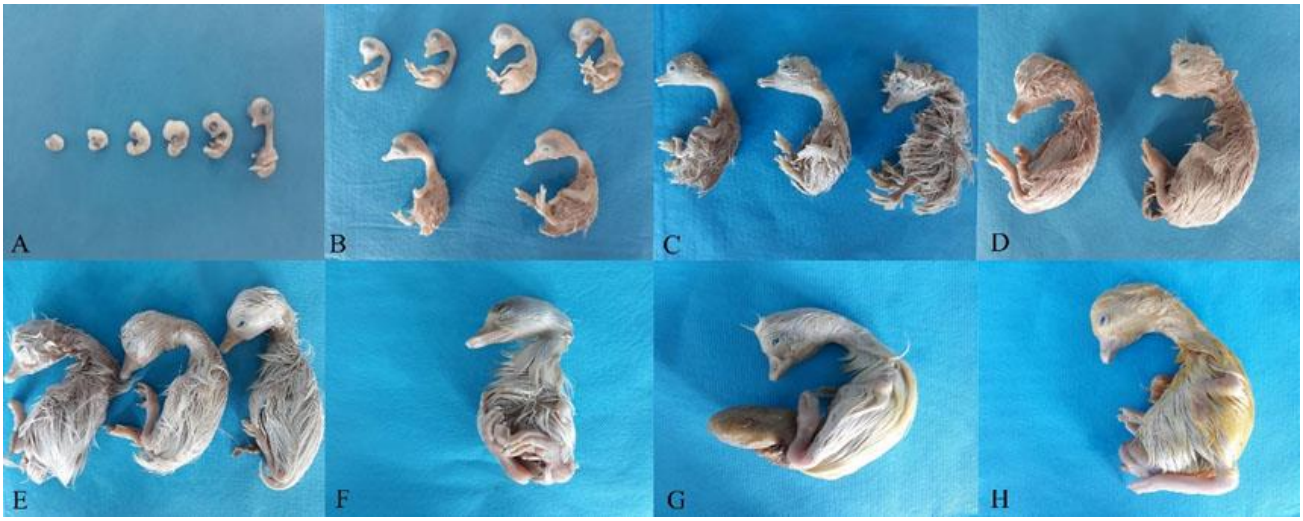

Figure 1. Geese (*Anser anser*) embryos in the hatching period (29 days). **A:** Days 6-11, **B:** Days 12-17, **C:** Days 18-20, **D:** Days 21-22, **E:** Days 23-25, **F:** Day 26, **G:** Day 27, **H:** Day 28

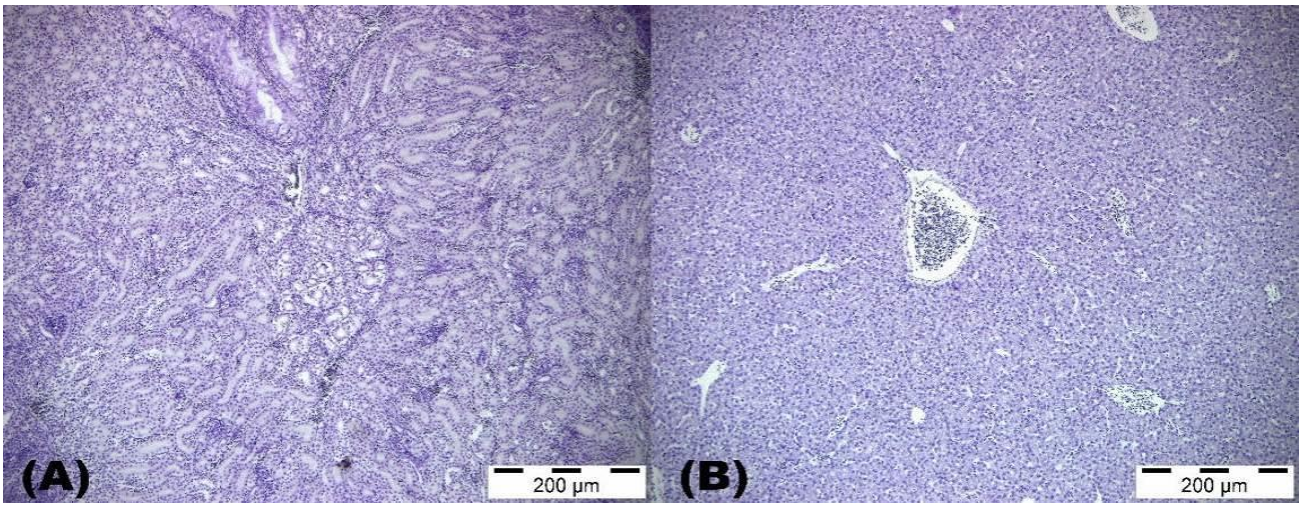

Figure 2. Negative control in kidney (**A**) and liver (**B**) tissues (Adult *Anser anser*), ×10 Avidin-biotin-peroxidase complex staining

RESULTS

Kidney

Tubule formation commenced by day 6 during the hatching period (Figure 3A). Mesonephric kidney was observed, along with the initiation of metanephric blastema formation on day 10 (Figure 3B). Degeneration of the mesonephric kidney began on days 16-17 (Figure 3C). By days 19-20, the cortex and medulla of the metanephric kidney began to differentiate (Figure 3D), with further distinction becoming more evident by day 25 (Figure 3E). In the post-hatching period, it was determined that the outer surface of the kidney is surrounded by a thin connective tissue capsule; this connective tissue sends extensions into the organ and shapes the lobules. There was a cortex and medulla in each lobe (Figure 3F). In the cortex, corpusculum renis, tubulus proximalis convoluta, tubulus distalis convoluta, perilobular ductus collectivus, and tubulus collectivus were seen (Figure 3F). In the medulla, thin and thick segments of Henle's loop, medullary ductus collectivus, and secondary branches of the ureter were seen (Figure 3G). It was determined that the corpusculum renis located in the cortex of the lobules belonged to the reptilian type nephron, and the corpusculum renis located close to the medulla belonged to the mammalian type nephron (Figure 3F, 3G, 3H). Hepatocyte growth factor (HGF) immunoreactivity was generally observed in the mesonephric tubules on hatching days 6-18, whereas in the metanephric kidney (pre-hatch and post-hatch, it was predominantly observed in the proximal tubules, with moderate expression in the ascending limb of the loop of Henle (Figure 4).

Insulin-like growth factor-I (IGF-I) immunoreactivity was generally observed in the mesonephric tubules on hatching

days 6-18, whereas it was mostly observed in the proximal tubules in the metanephric kidney (hatching and post-hatching periods (Figure 5). Fibroblast growth factor-2 (FGF-2) and transforming growth factor-α (TGF-α) immunoreactivity were generally observed in the mesonephric tubules and glomerulus on hatching days 6-18. In contrast, it was mostly observed in the proximal tubules, ascending limb of the loop of Henle, and secondary ureter sections in the metanephric kidney during hatching and post-hatching periods (Figures 6 and 7). There was no immunoreactivity associated with the four growth factors in the descending limb of the loop of Henle.

Liver

Areas formed by hematopoietic cells were observed in the liver at days 6-9 during the hatching period (Figure 8A). Hepatocytes, endothelial cells, and Kupffer cells were observed by day 6. The second lobe of the liver was more clearly distinguished from day 9 onward (Figure 8B). The radial arrangement of hepatocytes around the central vein was observed at day 10 (Figure 8C). The portal area and bile duct were observed on day 14 (Figure 8D). Fat storage started in the liver by post-hatching day 20, and this accumulation continued to decrease through post-hatching days 7 and 15 (Figure 8E, 8F).

It was determined that the histological structure of the liver was basically similar to the adult liver tissue starting from day 20 of hatching. It was observed that a thin connective tissue capsule surrounded the outer surface of the liver, and the lobule structure was not evident. In the portal area, the bile duct, hepatic artery, and interlobular vein were seen. It was determined that hepatocytes were polygonal in shape, most of them were dikaryotic, formed hepatic plaques in groups, and formed cords (Remark's cord) in groups of two, showing a radial arrangement around the central veins. It was observed that the sinusoids located between the Remark cords were covered by squamous endothelial cells, and there were Kupffer cells between these endothelial cells (Figure 5F). Additionally, in the present study, the PAS (+) reaction in hepatocytes, initially weak on day 13, became more prominent by day 15. In addition, the PAS (+) reaction was observed in Kupffer cells (Figure 9). Hepatocyte growth factor (HGF) immunoreactivity was observed in hepatocytes during the hatching and post-hatching periods, Kupffer cells on hatching days 6-14, and the bile duct by hatching day 22 (Figure 10). Insulin-like growth factor-I (IGF-I) immunoreactivity was observed in hepatocytes in the hatching and post-hatching periods, hematopoietic cells on hatching days 6-14, the bile duct, and the connective tissue in the portal area during the post-hatching period (Figure 11). Fibroblast growth factor-2 (FGF-2) and transforming growth factor-α (TGF-α) immunoreactivities were observed in hepatocytes in the hatching and post-hatching periods, hematopoietic cells on hatching days 6-14, Kupffer cells on hatching days 6-10, and connective tissue in the portal area from hatching day 22 (Figures 12 and 13).

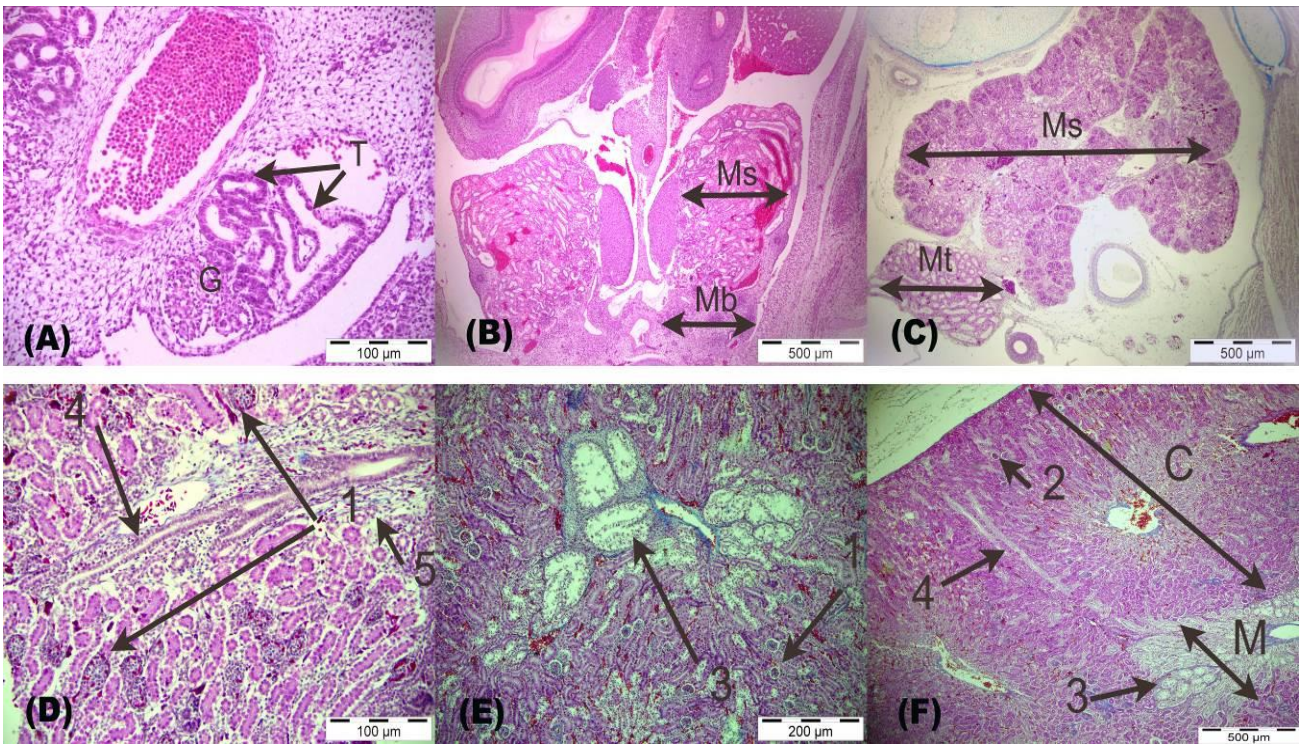

Figure 3. General view of the goose kidney in the pre-hatch (29 days) and post-hatch period. **A:** Day 6 (×20), **B:** Day 10 (×4), **C:** Day 17 (×4), **D:** Day 25 (×20), **E:** Post-hatching on day 15 (×10), **F:** Adult (×4), **G:** Glomerulus, **T:** Tubules, **Ms:** Mesonephros, **Mb:** Metanephric blastema, **Mt:** Metanephros, **M:** Medulla, **C:** Cortex, **1:** Mammalian type glomerulus, **2:** Reptilian type glomerulus, **3:** Medullary ductus collectivus, **4:** Peri-lobulary ductus collectivus, **5:** Ascending limb of loop of Henle. **A** and **B**: H&E staining, **C, D, E, F, G,** and H: Crossmonn's modified triple staining.

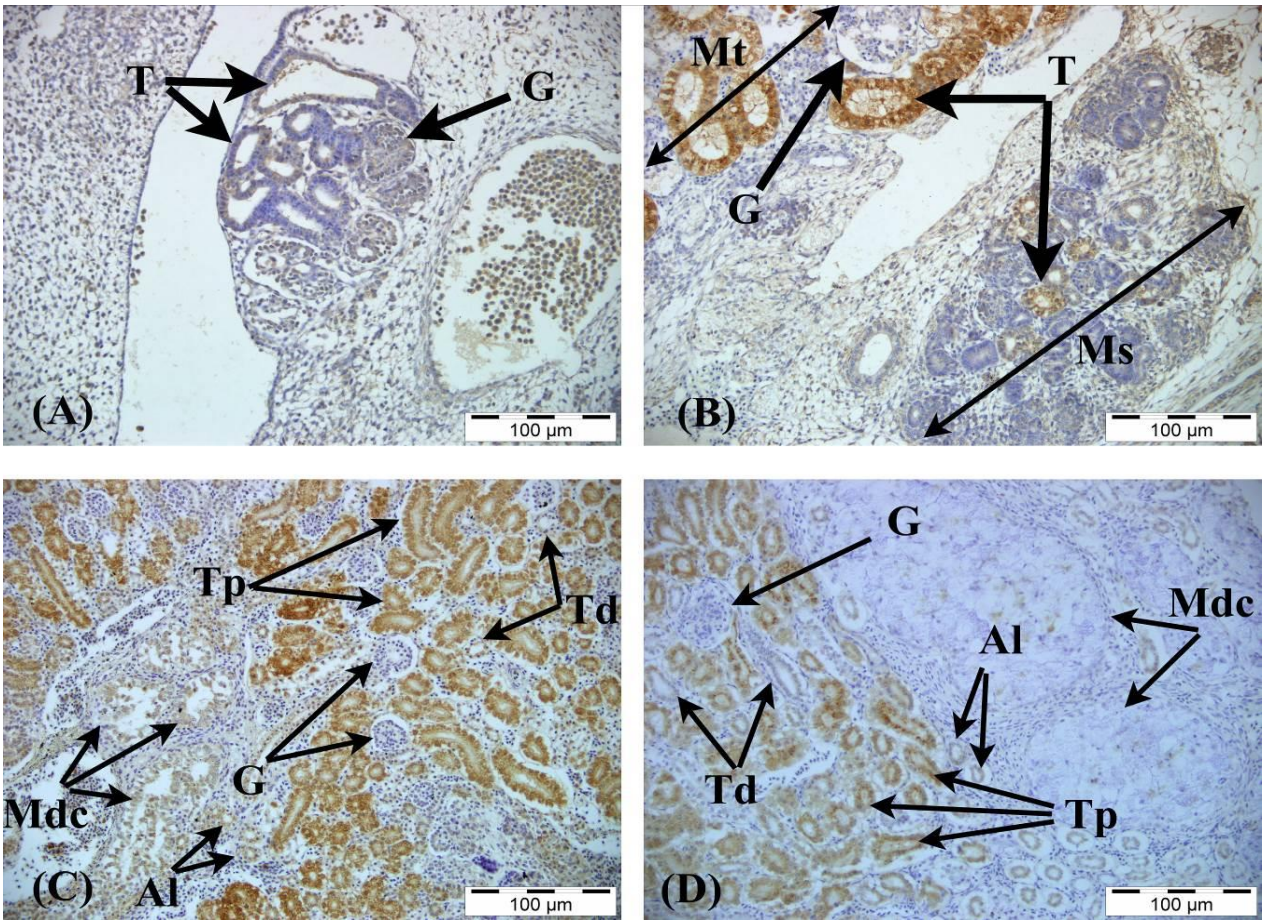

Figure 4. General view of HGF immunoreactivity in goose (*Anser anser*) kidney during incubation (29 days) and post-incubation period. **A:** Day 6, **B:** Day 14, **C:** Day 22, **D:** Adult, **G:** Glomerulus, **T:** Tubules, **Ms:** Mesonephros, **Mt:** Metanephros, **Tp:** Proximal tubules, **Td:** Distal tubules, **Al:** Ascending limb of loop of Henle. **Mdc:** Medullary ductus collectivus (×20).

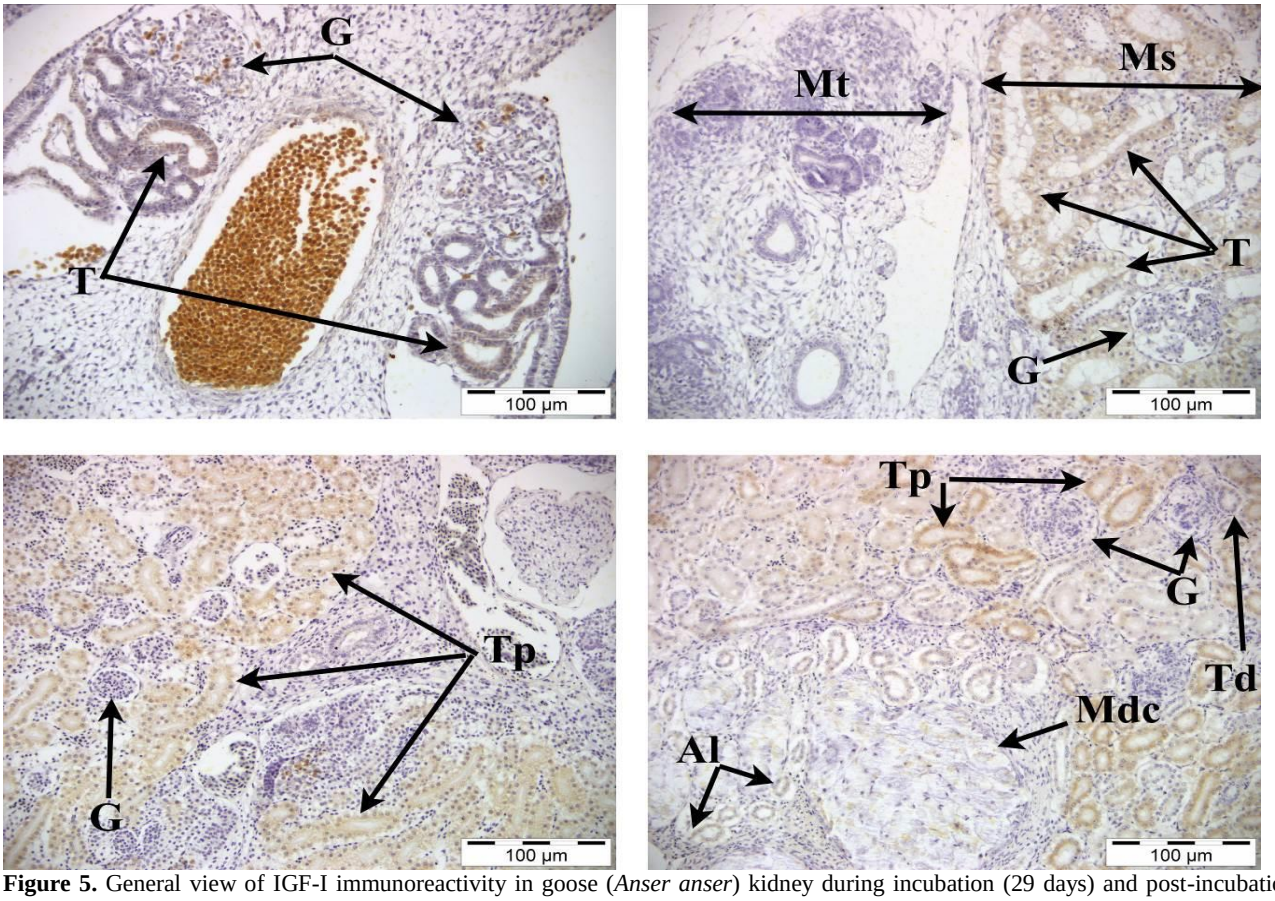

Figure 5. General view of IGF-I immunoreactivity in goose (*Anser anser*) kidney during incubation (29 days) and post-incubation period. **A:** Day 6, **B:** Day 14, **C:** Day 22, **D:** Adult, **G:** Glomerulus, **T:** Tubules, **Ms:** Mesonephros, **Mt:** Metanephros, **Tp:** Proximal tubules, **Td:** Distal tubules, **Al:** Ascending limb of loop of Henle. **Mdc:** Medullary ductus collectivus (×20).

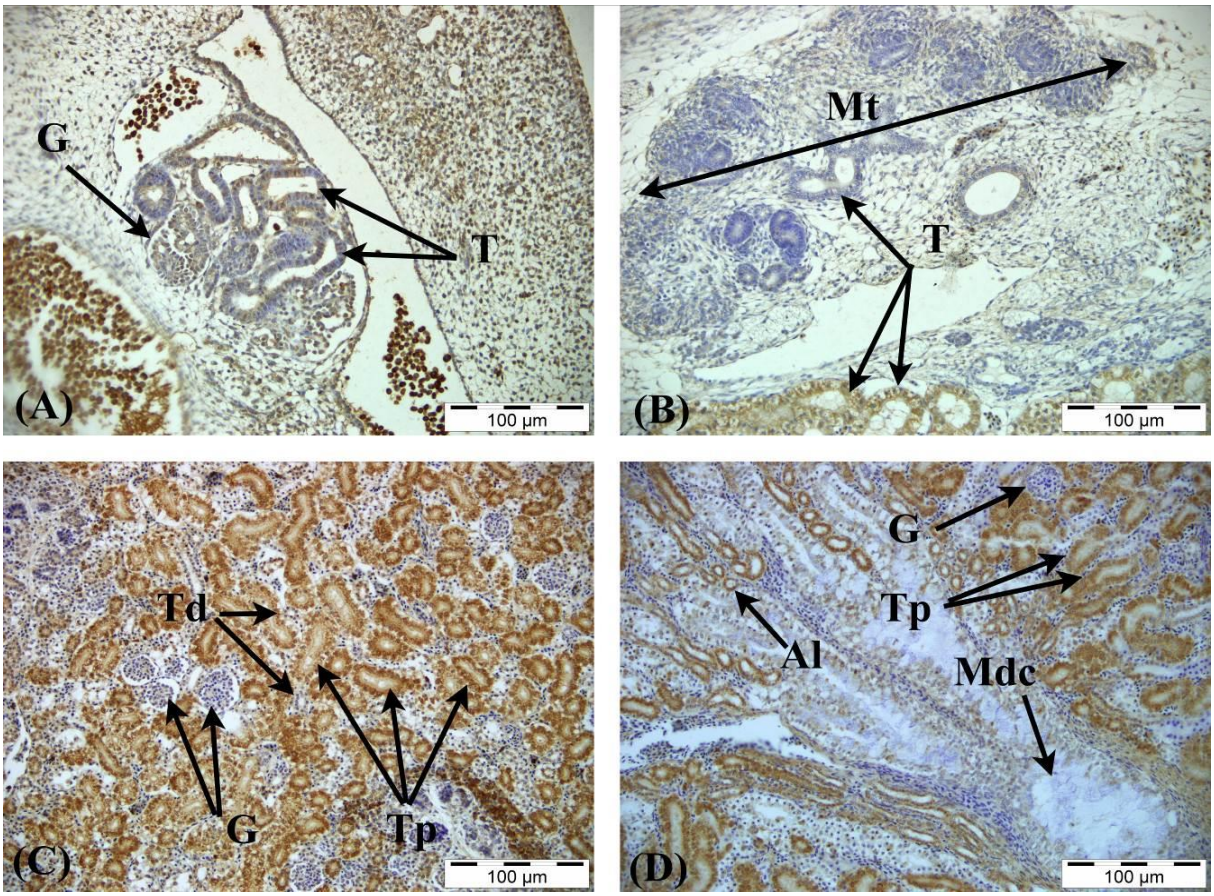

Figure 6. General view of FGF-2 immunoreactivity in goose (*Anser anser*) kidney during incubation (29 days) and post-incubation period. **A:** Day 6, **B:** Day 14, **C:** Day 22, **D:** Adult, **G:** Glomerulus, **T:** Tubules, **Mt:** Metanephros, **Tp:** Proximal tubules, **Td:** Distal tubules, **Al:** Ascending limb of loop of Henle. **Mdc:** Medullary ductus collectivus (×20).

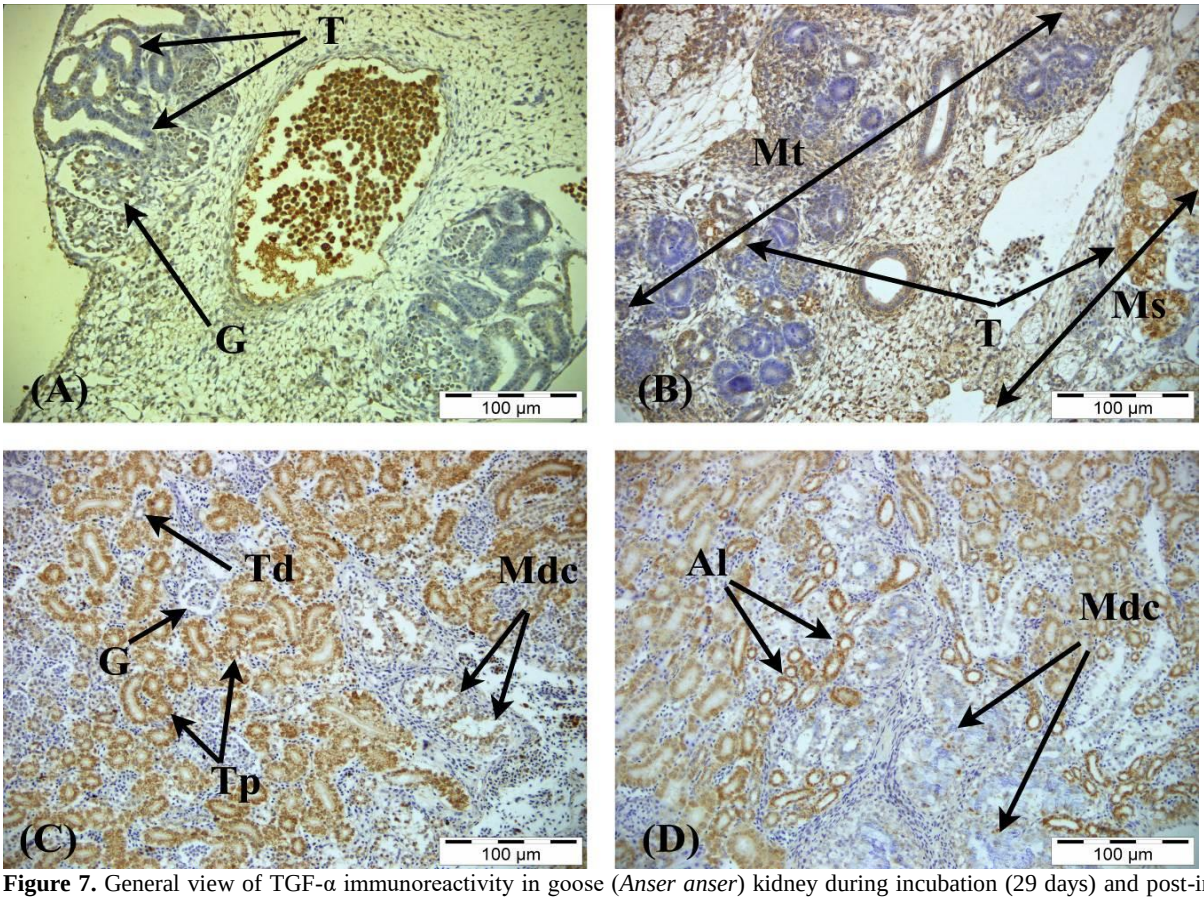

Figure 7. General view of TGF-α immunoreactivity in goose (*Anser anser*) kidney during incubation (29 days) and post-incubation period. **A:** Day 6, **B:** Day 14, **C:** Day 22, **D:** Adult, **G:** Glomerulus, **T:** Tubules, **Ms:** Mesonephros, **Mt:** Metanephros, **Tp:** Proximal tubules, **Td:** Distal tubules, **Al:** Ascending limb of loop of Henle. **Mdc:** Medullary ductus collectivus (×20).

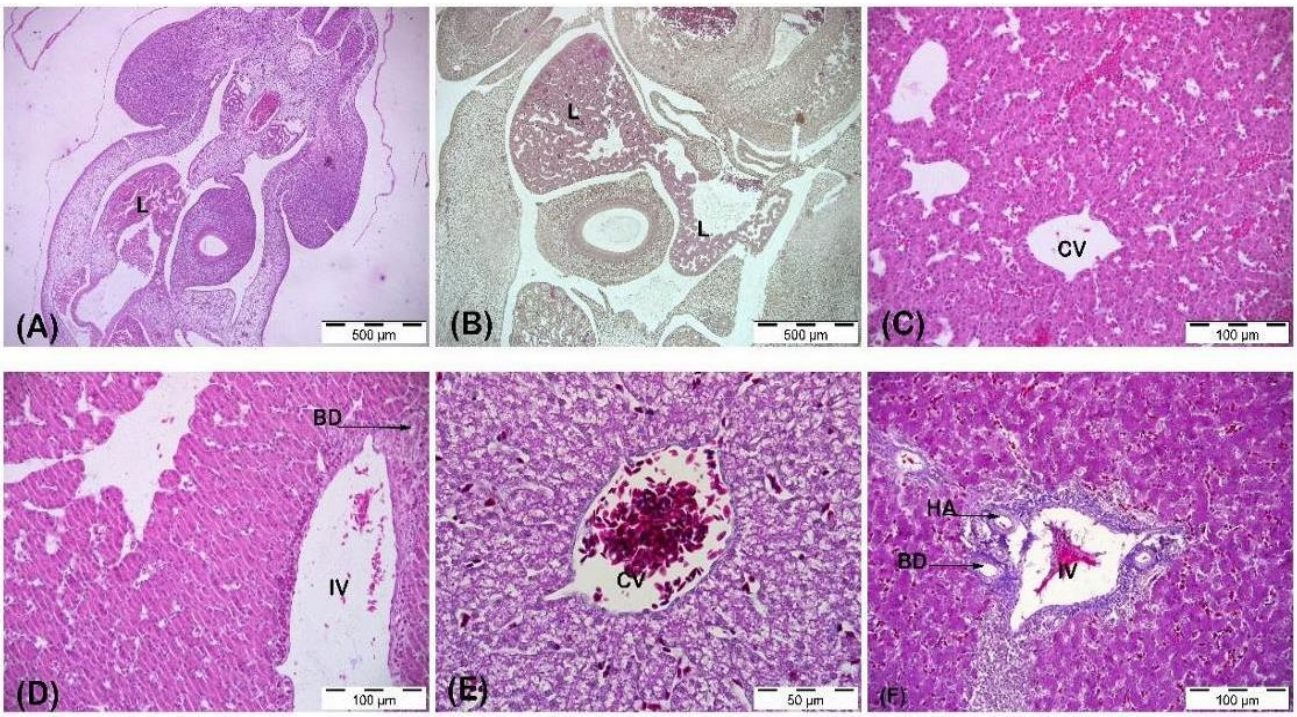

Figure 8. General view of the goose (*Anser anser*) liver in the pre-hatch (29 days) and post-hatch period. **A:** Day 6 (×4), **B:** Day 9 (×4), **C:** Day 10 (×20), **D:** Day 14 (×20), **E:** Day 23 (×40), **F:** Adult (×20). **L:** Liver, **CV:** Central vein, **IV:** Interlobular vein, **BD:** Bile duct, **HA:** Hepatic artery. **A, C,** and **D:** H&E staining, **B, E,** and **F:** Crossmonn's modified triple staining.

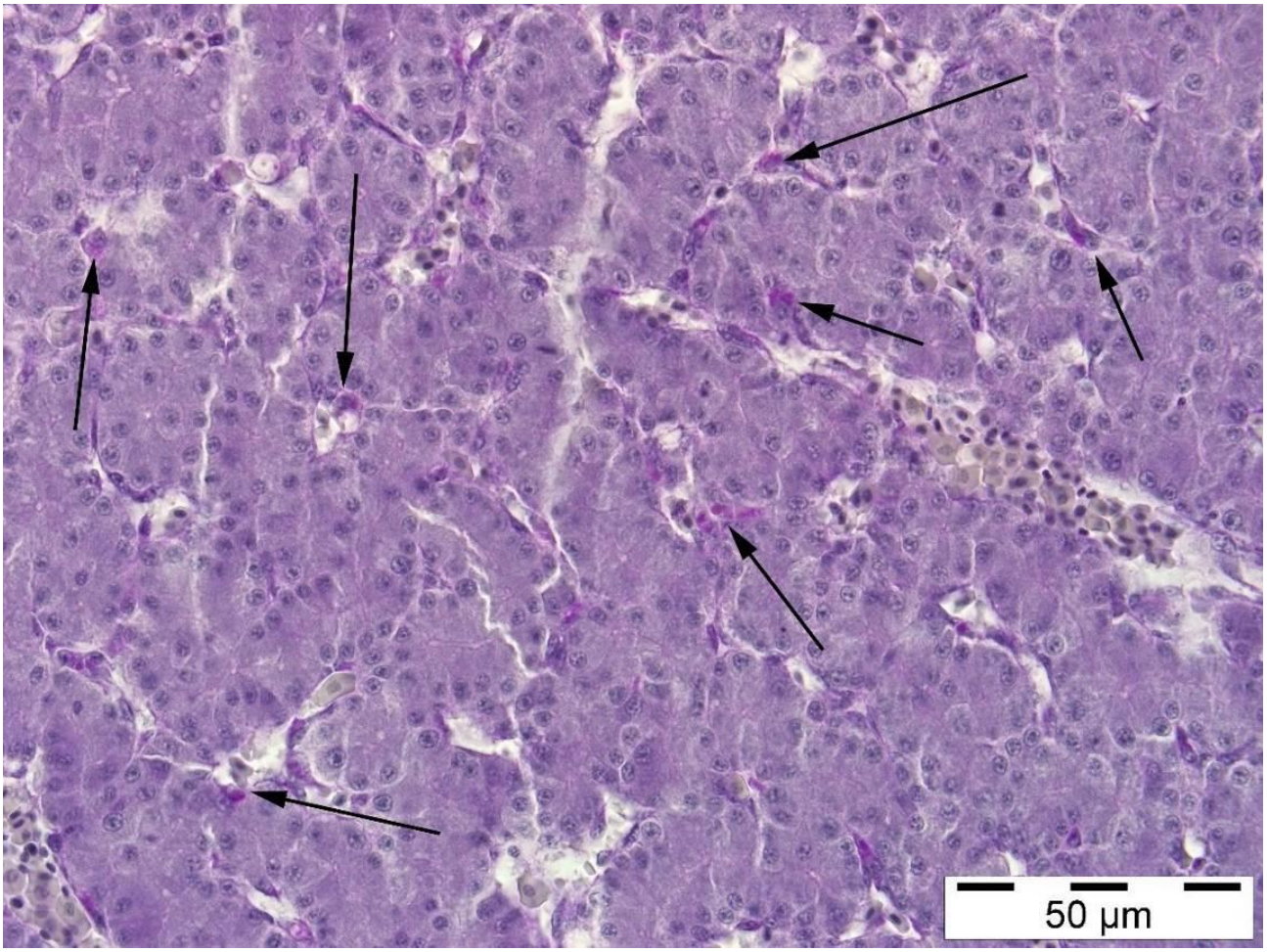

Figure 9. Periodic acid-Schiff (PAS) staining in geese (*Anser anser*), hatching day 15. Arrows: PAS (+) cells (×40).

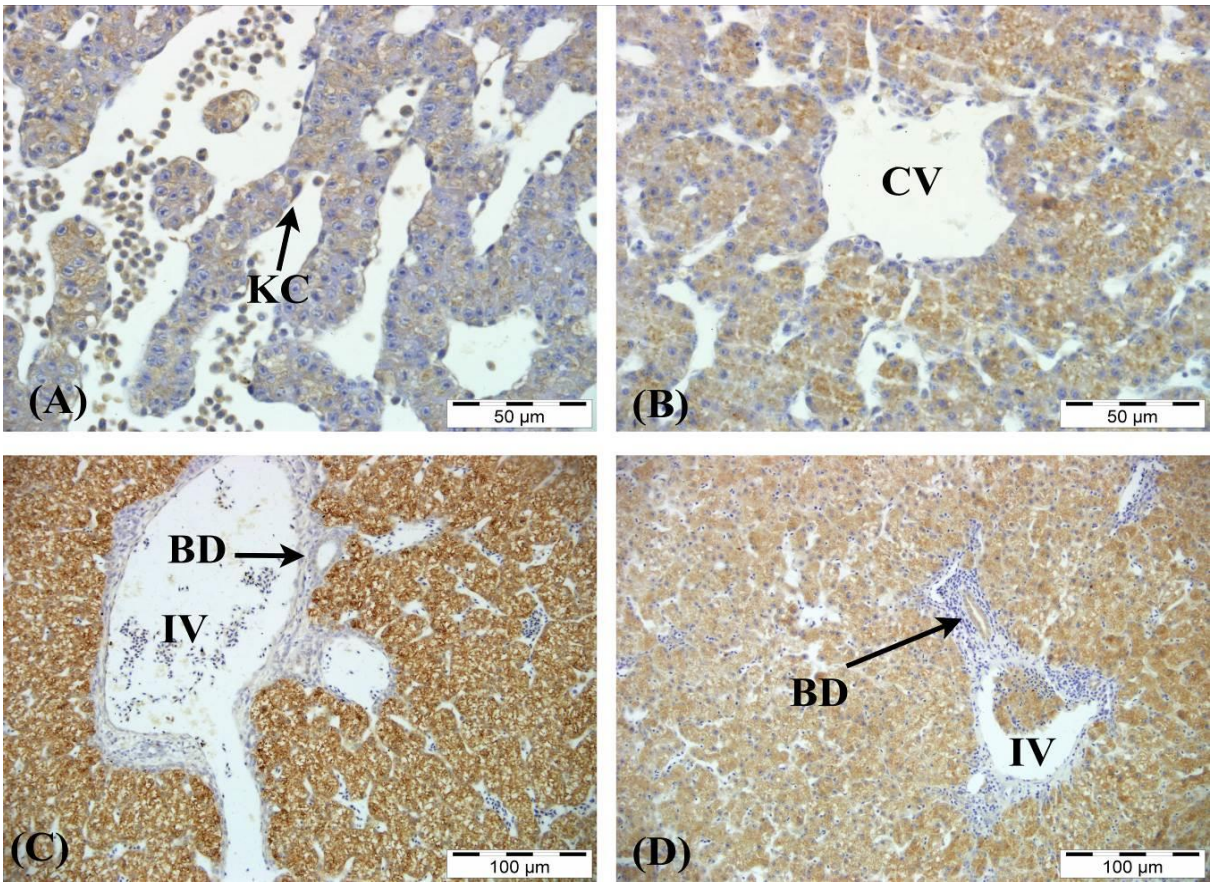

Figure 10. General view of HGF immunoreactivity in goose (*Anser anser*) liver during incubation (29 days) and post-incubation period. **A:** Day 6 (×40), **B:** Day 14 (×40), **C:** Day 22 (×20), **D:** Adult (×20), **KC:** Kupffer cells, **HC**: Hematopoietic cells, **CV:** Central vein, **IV:** Interlobular vein, **BD:** Bile duct, and **CT:** Connective tissue.

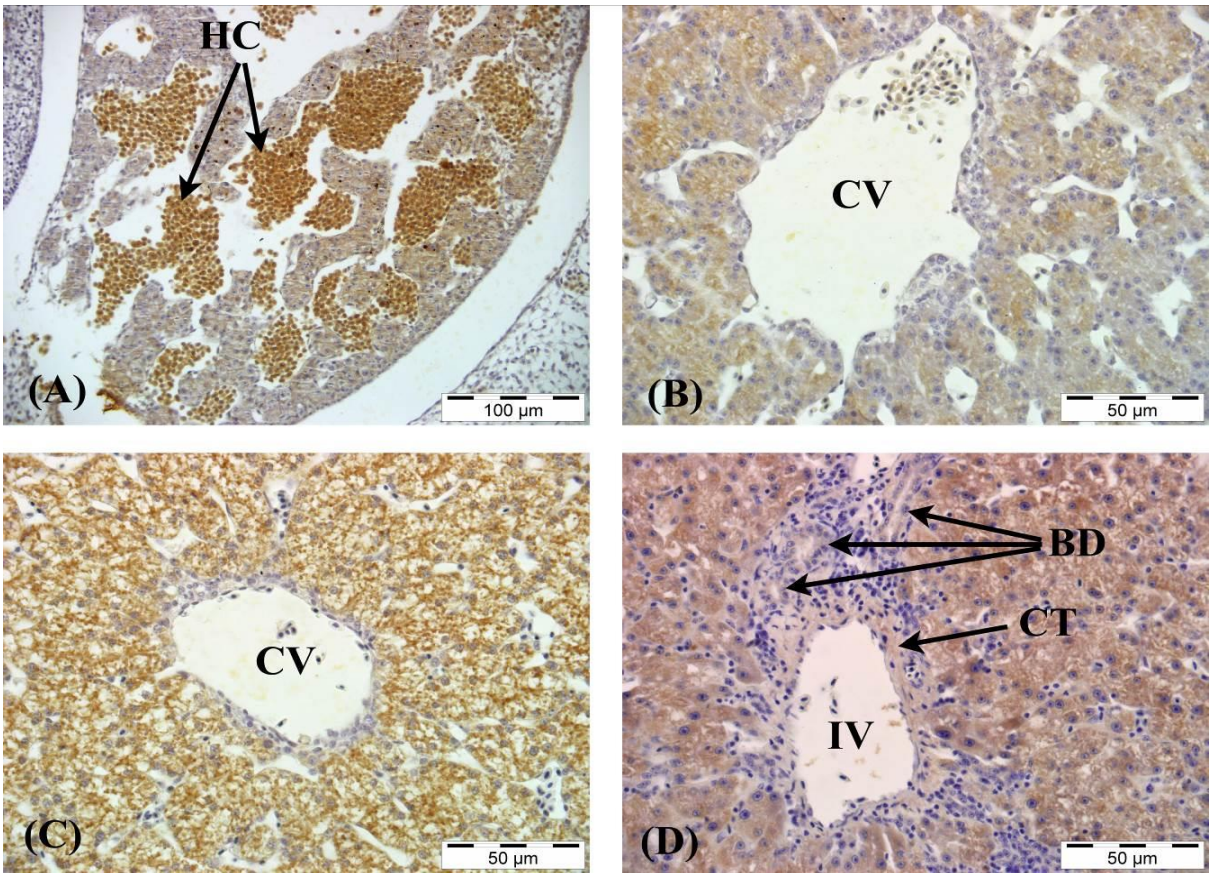

Figure 11. General view of IGF-I immunoreactivity in goose (*Anser anser*) liver during incubation (29 days) and post-incubation period. **A:** Day 6 (×20), **B:** Day 14 (×40), **C:** Day 22 (×40), **D:** Adult (×40), **KC:** Kupffer cells, **HC**: Hematopoietic cells, **CV:** Central vein, **IV:** Interlobular vein, **BD:** Bile duct, and **CT:** Connective tissue.

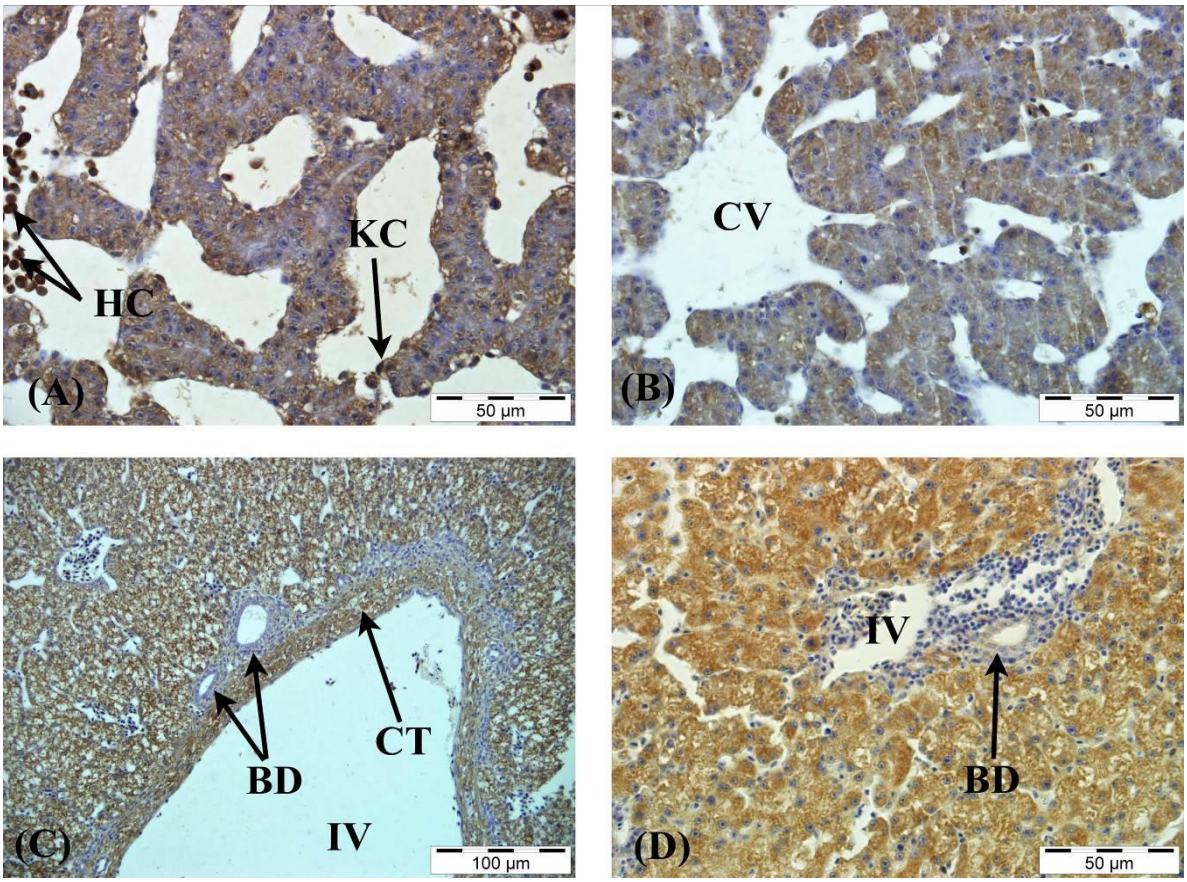

Figure 12. General view of FGF-2 immunoreactivity in goose (*Anser anser*) liver during incubation (29 days) and post-incubation period. **A:** Day 6 (×40), **B:** Day 14 (×40), **C:** Day 22 (×20), **D:** Adult (×40), **KC:** Kupffer cells, **HC**: Hematopoietic cells, **CV:** Central vein, **IV:** Interlobular vein, **BD:** Bile duct, and **CT:** Connective tissue.

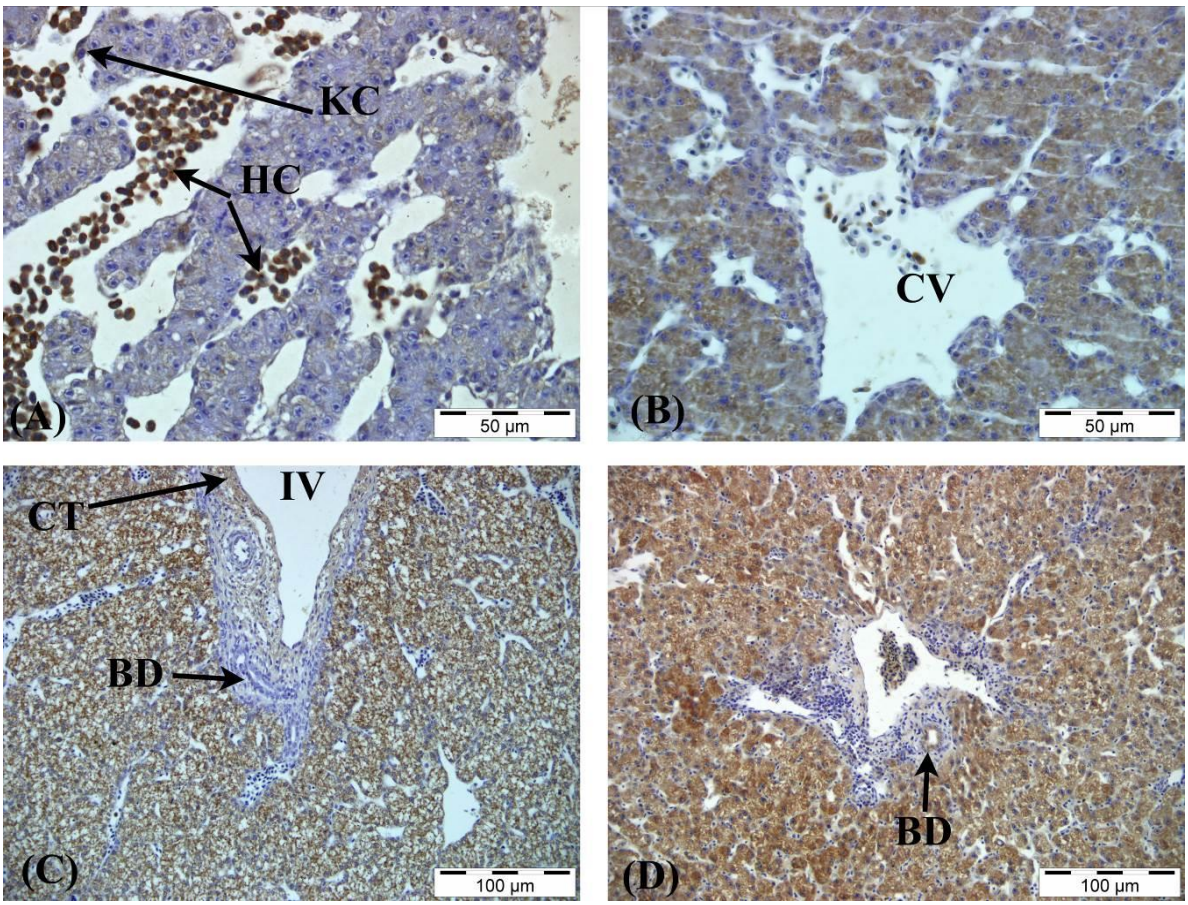

Figure 13. General view of TGF-α immunoreactivity in goose (*Anser anser*) liver during incubation (29 days) and post-incubation period. **A:** Day 6 (×40), **B:** Day 14 (×40), **C:** Day 22 (×20), **D:** Adult (×20), **KC:** Kupffer cells, **HC**: Hematopoietic cells, **CV:** Central vein, **IV:** Interlobular vein, **BD:** Bile duct, and **CT:** Connective tissue.

DISCUSSION

Kidney

The mesonephric kidney in chicken embryos morphologically formed on days 3-4, became functional on days 5-11, and degenerated into the metanephros on day 15 (Neuhaus and Hollemann, 2009; Bolin and Burggren, 2013). In the present study, the mesonephric kidney was observed on hatching day 6, and the metanephric blastema and the mesonephric kidney were formed on day 10. The general structure of the metanephric kidney began to fully form by day 14. The mesonephric kidney was observed on day 15 in chickens (Neuhaus and Hollemann, 2009; Bolin and Burggren, 2013) and on day 25 in geese. Histological studies have been conducted on the kidneys of poultry animals such as quail (Ahmad Alabdallah et al., 2021), goose (Taşçı et al., 2020), chicken, and duck (Abood et al., 2014). In these studies, cortex and medulla were identified in each lobe; corpusculum renis, proximal convoluta tubule, distal convoluta tubule, perilobular collectivus, and collectivus tubule in the cortex; thin and thick segments of Henle's loop, medullary collectivus ductus, and secondary branches of the ureter were identified in the medulla. In the present study, the histological structure of the kidney tissue of adult geese was consistent with that reported in previous studies (Abood et al., 2014; Taşçı et al., 2020; Ahmad Alabdallah et al., 2021).

HGF

Hepatocyte growth factor (HGF) immunoreactivity was reported in collecting tubules on day 17 of development in fetal rat kidneys (Defrances et al., 1992). HGF is produced in renal epithelial cells in mice kidney (Deprem et al., 2020) and plays an important role in cell growth and differentiation as well as embryogenesis (Davies, 2001; Matsumoto and Nakamura, 2001; Liu, 2002). In the present study, HGF immunoreactivity was observed in the mesonephric kidney tubules and the metanephric kidney; however, intense immunoreactivity was observed in the proximal tubules.

IGF-I

While serum IGF-I was detected in turkey (McMurtry et al., 1996) and chick embryos (Robcis et al., 1991), IGF-I was determined immunohistochemically in embryonic human and rat kidney (Coppola et al., 2009; Gurevich et al., 2021) and adult rat kidneys (Saltan and Aslan, 2017). IGF-I, produced in kidney tissue, plays a crucial role in bovine embryonic development, enhances cell proliferation (Makarevich and Markkula, 2002), and regulates the differentiation of germ layers in the early stages of embryogenesis as well as the organogenesis process (Mercola and Stiles, 1988). In the present study, IGF-I showed intense immunoreactivity in the hatching and post-hatching periods, especially in the proximal tubules.

FGF-2

Fibroblast growth factor-2 (FGF-2) was immunohistochemically detected in fetal (Cancilla et al., 1999) and adult rat kidneys (Floege et al., 1992). Fibroblast growth factor-2 was found to be expressed in various epithelial structures, such as the ureter, proximal tubules, and parietal epithelium of the glomerulus, in the fetal human kidney (Drummond et al., 1998). It was also suggested that FGF-2 stimulated the development of renal tubules and capillary growth in the embryonic process (Carlson, 2018), triggered the migration and proliferation of endothelial cells, and promoted the mitogenesis of smooth muscle cells and fibroblasts that induced the development of large vessels (Beenken and Mohammadi, 2009). In the present study, FGF-2 demonstrated intense immunoreactivity in the proximal tubules and ascending limb of the loop of Henle, as well as in sections of the secondary ureter.

TGF-α

Carev et al. (2008) reported that TGF-α immunoreactivity was observed in all mesonephric structures in the fetal human kidney at week 7 of gestation, but was more intense in the tubules. Intense TGF-α immunoreactivity in the proximal tubules of fetal human kidneys (Carev et al., 2008) was consistent with this study on geese. Previous studies on chick embryos (Diaz Ruiz et al., 1993) and adult chicken kidneys (Diaz Ruiz et al., 1996) reported TGF-α immunoreactivity in the distal tubules, with no observed immunoreactivity in glomeruli, proximal tubules, or the descending and ascending limbs of the loop of Henle. In the present study, TGF-α immunoreactivity was observed in all tubules and glomeruli in the mesonephric kidney. These results are consistent with the studies of Diaz Ruiz et al. (1993) and Diaz Ruiz et al. (1996) only in terms of distal tubules. Transforming growth factor-α has been reported to be present in self-renewing tissues, supporting new cell formation and development (Yetkin and Çelebi, 2001) and playing an important role in embryonic and fetal development (Burgess, 1989; Schultz et al., 1994). In the present study, intense immunoreactivity of TGF-α in renal tubules (especially in the proximal tubules and ascending limb of the loop of Henle) and in sections of the secondary ureter during the hatching and post-hatching periods suggests its crucial role in renal

development. Four growth factors examined in the hatching and post-hatching periods did not exhibit immunoreactivity in the descending limb of the loop of Henle.

Liver

Çöllü and Gürcü (2017) have indicated that the chicken liver forms in the cardio-hepatic region, with two lobes detected on hatching day 6 and the third lobe on day 7. Similar to chicks, the liver is located in the cardio-hepatic region in goose embryos. Unlike the chicken embryos, the second lobe could be distinguished on days 8-9, but the third lobe could not be observed. In the present study, the general histologic structure of the liver tissue of adult geese was similar to that reported in the Buteo buteo and Alectoris Chukar (Taşçı et al., 2018; Kara et al., 2019).

In poultry, fat synthesis is 20 times higher in hepatic tissue than in adipose tissue, unlike in mammals, leading to periodic fat accumulation in avian livers (Uni et al., 2012; Erhan and Ergün, 2018; Zaefarian et al., 2019). Consistent with previous studies, fat droplet formation in liver hepatocytes began around day 20 in the present study, peaking by days 25-28 (Uni et al., 2012; Erhan and Ergün, 2018; Zaefarian et al., 2019). In the present study, adiposity continued to decrease on post-hatching days 7, 15, and 30. In poultry, the embryo cannot obtain glucose from the maternal source and actively undergoes gluconeogenesis during developmental stages (Erhan and Ergün, 2018). As poultry store small amounts of carbohydrates in their livers, maintaining glucose concentration depends on gluconeogenesis (Erhan and Ergün, 2018). A study on partridge embryos (Hashemnia et al., 2015) reported PAS (+) reaction in hepatocytes cytoplasm on day 13 of development, whereas another study on chicken embryos (Doaa et al., 2013) reported PAS reactions beginning on day 5 and increasing by day 13. In the present study, the PAS (+) reaction in hepatocytes, initially weak on day 13, became more prominent by day 15. In addition, inconsistent with previous studies, PAS (+) reaction was observed in Kupffer cells and hepatocytes (Doaa et al., 2013; Hashemnia et al., 2015).

HGF

Iida et al. (2003) reported that HGF immunoreactivity was absent in fetal and neonatal rat livers but present in hepatocytes around the central vein during the post-hatching period. Wolf et al. (1991) reported that HGF immunoreactivity was moderate in Kupffer cells and the vascular endothelium in the rat liver, whereas there was no reaction in hepatocytes or bile ducts. It has been suggested that HGF played an important role in cell growth and differentiation as well as embryogenesis (Matsumoto and Nakamura, 2001; Davies, 2001; Liu, 2002) and that it was expressed in the liver by certain cells, including endothelial, Kupffer, and Ito cells (Yin et al., 2013; Thompson et al., 2015). In the present study, HGF immunoreactivity was observed in Kupffer cells during the initial days of the hatching period (days 6-14), in hepatocytes on all hatching and post-hatching days, and in epithelial cells of the bile duct on hatching day 22, indicating that HGF plays an important role in liver development.

IGF-I

Ralphs et al. (1990) observed similar immunohistochemical distribution of IGF in 13-day-old rat embryos and 20-28-day-old chick embryos, primarily in non-chondrogenic connective tissue, heart, smooth muscle, and peripheral and central nervous systems. Saltan and Aslan (2017) investigated IGF-I immunoreactivity in the livers of adult rats, reporting a reaction primarily in hepatocytes and the bile duct. Consistent with these findings, immunoreactivity was observed in hepatocytes and bile ducts in adult geese during the post-hatching period. IGF-I synthesis mainly occurs in the liver (Le Roith, 1997; Le Roith et al., 2001) and promotes increased mitotic activity during the post-hatching period (Owino et al., 2001). In the present study, IGF-I immunoreactivity was observed in hematopoietic cells early in the hatching period (days 6-14), hepatocytes in the hatching and post-hatching periods, and connective tissue in the portal area during the post-hatching period, highlighting its important role in liver development.

FGF-2

Gonzalez et al. (1990) examined 18-day-old rat fetuses and found lower FGF-2 immunoreactivity in the fetal liver compared with other tissues, and observed immunoreactivity only in the capsule and vessels but not in hepatocytes. It has been suggested that FGF-2 promotes liver development and stimulates capillary growth (Carlson, 2018), with FGF signaling playing an important role in liver differentiation (Zhang et al., 2004; Shin et al, 2007). In the present study, intense FGF-2 immunoreactivity was observed in liver hepatocytes during the hatching and post-hatching periods.

TGF-α

In an immunohistochemical study in adult rats, intense immunoreactivity of TGF-α was observed in the epithelium of newly formed bile ducts, with no immunoreactivity in hepatocytes (Alison et al., 1993). In the present study, different intensities of TGF-α immunoreactivity were observed in hepatocytes during hatching and post-hatching periods, whereas

no immunoreactivity was observed in the bile duct during the hatching period, contrary to the findings by Alison et al. (1993). It has been reported that TGF-α acts as a mitogen for hepatocytes and is primarily produced by hepatocytes undergoing DNA replication (Webber et al., 1994); supports new cell formation in the tissues where it is present (Yetkin and Çelebi, 2001); and directs the growth and differentiation processes of organogenesis (Mercola and Stiles, 1988). In the present study, intense TGF-α immunoreactivity was observed in liver hepatocytes during the hatching and post-hatching periods.

CONCLUSION

As a result of this study, the expressions of HGF, IGF-I, FGF-2, and TGF-α were assessed immunohistochemically in the goose kidney and liver during the hatching and post-hatching periods. Upon general evaluation, FGF-2 exhibited the most intense immunoreactivity in the liver and kidney, whereas IGF-I showed the least immunoreactivity. The concentration of the studied growth factors in the proximal tubules in the kidney and hepatocytes in the liver suggests that these organs are the main expression sites for HGF, IGF-I, FGF-2, and TGF-α. These findings highlight the significance of proximal tubules in reabsorption and the special metabolic tasks carried out by hepatocytes. It is thought that this study will contribute to the examination of the expression profiles of growth factors according to developmental periods using advanced molecular methods in embryonic development studies in various poultry species.

DECLARATIONS

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Authors' contributions

Şahin Aslan and Dilem Gülece Ermutlu designed the study. Dilem Gülece Ermutlu conducted the histological examination, interpretation, and preparation of the manuscript. All authors read and approved the final version of the manuscript to be published.

Conflict of interests

The authors have not declared any conflicts of interest.

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Ethical considerations

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Ethical issues, including consent to publish, misconduct, fabrication of data, and redundancy, have been checked by all authors. The authors did not use any AI applications for writing the full text of this article.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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