

Steps of the Pancreatic Differentiation in the Grass Snake (*Natrix natrix*) Embryos

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Abstract : The pancreas is an important organ present in all vertebrate species. It contains two different tissues, exocrine and endocrine, that act as two glands in one. The development and differentiation of the pancreas in reptiles is poorly known in comparison to other vertebrates. Therefore, the aim of this study was to investigate the particular steps concerning the differentiation of the pancreas in the grass snake (*Natrix natrix*) embryos. For this, histological methods (including hematoxylin and eosin, and Heidenhain's AZAN staining), transmission electron microscopy and three-dimensional (3D) reconstructions from serial paraffin sections were used. The results of this study indicated that the first step of pancreas development in *Natrix* was the connection of the two pancreatic buds: dorsal and ventral one. Then, duct walls in both buds started to be remodeled from the multilayered to single-layered epithelium. This remodeling started in the dorsal bud and was simultaneously with the differentiation of the duct lumens which occurred by the cavitation. During this process, the cells that had no contact with the mesenchyme underwent cell death named anoikis. These findings indicated that the walls of ducts in the embryonic pancreas of the grass snake were initially formed by the abundant principal and single endocrine cells. Later the basal and goblet cells differentiated. Among the endocrine cells, as the first the B and A cells differentiated, then the D and PP cells. The next step of the pancreatic development was the withdrawing of the endocrine cells from the duct walls to form the pancreatic islets. The endocrine cells and islets were found only in the dorsal part of the pancreas in *Natrix* embryos what is different than in other vertebrate species. The islets were formed mainly by the A cells. Simultaneously, with the differentiation of the endocrine pancreas, the acinar tissue started to differentiate. The source of the acinar cells were pancreatic ducts similar as in other vertebrates. The acini formation began at the proximal part of the pancreas and went towards the caudal direction. Differentiating pancreatic ducts developed into the branched system that can be divided into extralobular, intralobular, and intercalated ducts, similarly as in other vertebrate species. However, the pattern of branching was different. In conclusions, particular steps of the pancreas differentiation in the grass snake were different than in other vertebrates. It can be supposed that these differences are related to the specific topography of the snake's internal organs and their taxonomy position. All specimens used in the study were captured according to the Polish regulations concerning the protection of wild species. Permission was granted by the Local Ethics Commission in Katowice (41/2010; 87/2015) and the Regional Directorate for Environmental Protection in Katowice (WPN.6401.257.2015.DC).

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