SUMMARY

Keywords: *quail, Coturnix japonica, morphology, ovary, oviduct, neurotrophins, immunohistochemistry*

Studies on the development of biological systems have expanded using animal models, always to the elucidation of biochemical and biophysical aspects of ontogenetic processes. Embryology seeks to capture the biochemistry changes at the molecular level, underlying the development and activity of the embryonic body, to thereby reveal fundamental mechanisms of ontogenetic development. Thus, different species as birds have been used in particular for the study of embryonic development. Currently the Japanese quail is used as an experimental model.

Expansion of therapeutic interventions for the prevention of infertility in both women and domestic animals depends largely on the reproductive age. Thus, the component of the reproductive axis plays a role in the decline of fertility. As a result, we achieved avian models that should prove the effectiveness of the mechanisms of natural defence against damage to the reproductive process.

Knowledge of the morphology of female reproductive apparatus has great importance in economic evaluation of the quail, regarding such aspects as the incubation period, the growth and the increase of the percentage of hatching. In recent years the involvement of neurotrophins family in the process of breeding has been discovered, their role being portrayed in the ovary and oviduct different species of mammals. Currently the expression and their role in the reproductive system in poultry is not known. The aim of this thesis is to investigate the presence of neurotrophines in the ovary and quail's oviduct following their role in the development and functioning of the reproductive system.

The doctoral thesis entitled "Morphology and citochemistry of quail ovary and oviduct ***(Coturnix coturnix japonica)"*** has been developed within the framework of the Doctoral School of Iaşi's Faculty of veterinary medicine of the University of agricultural sciences and veterinary medicine *"Ion Ionescu de la Brad"* , Iasi, as well as at the University of Naples, Italy, during the four years of study, from 1st October 2010 to 1st October 2014 and is structured in accordance with current legal provisions in two parts: part I-a entitled "*Actual state of knowledge"*, which

includes the 44 pages and represent 33% and part II entitled "*Personal Contribution",* which is expanded on 87 pages., representing 67%.

Part I-the is a study conducted by consulting 255 titles and it is structured in three chapters, in which they is synthesized information from the literature consulted on the subject of the thesis. In this part we used 20 figures to illustrate the aspects studied and 2 tables to detail the information presented.

In the first chapter, entitled "*The morphology of quail reproductive system*", the Japanese quail is presented as model in research studies. In this chapter there are synthesized the development stages in relation to other species of birds. The development of the ovary and oviduct in quails is described highlighting the histological aspects and followed by macroscopic and microscopic description of the genital apparatus. Chapter II entitled "*Neurotrophins family*" describes the biochemical profile, his phylogenesis and their mode of action, focusing on biological activity in the reproductive system in particular. In chapter III, "*Mechanism of the egg formation*" physiological changes are specified in relation to the study of hormones and neurotrophins.

Part II "*Personal Contributions"* consists of 4 chapters (cap. IV-VII). Chapter IV presents the purpose and objectives of the thesis, the following chapters (V and VI) presenting the materials and work methods, research results, discussions, interpretations and partial conclusions. In Chapter VII, the final conclusions are presented, which synthesize the results.

In chapter IV, with the heading "*Aim and objectives* " the goal and the main objectives of the doctoral thesis are presented with the necessary activities needed to achieve them. The main goal of the thesis is the description of the morphology of the ovary and oviduct in quails (*Coturnix coturnix japonica)*, from sex differentiation to the peak of the laying period in quail. Morphological and citochemical aspects of genital development at this bird specialized in produce of eggs, were pursued to highlight the morphofunctional particularities. Another objective was to study the presence of neurotrophins family in the genital apparatus using by immunohistochemistry and immunofluorescence exams. Through this study we identified the presence of neurotrophins and their specific receptors in the ovary and oviduct, searching for their role in the reproductive system in fowl. Hormonal study was correlated with the development of the genitalia in quail.

Research from chapter V, entitled *"Optical microscopy of the ovary and oviduct in quail (Coturnix coturnix japonica)"* were achieved by sampling from a total of 230 cases: 122 embryos, 46 quails aged between 1 and 44 days; also 62 adult quails. The research material was provided by the Biobase U.Ş.A.M.V. Iaşi. Macroscopic images were made, and the eggs and the

embryos were weighted to highlight the embryonic development. We measured the histological structures of the ovary and oviduct in order to highlight their development. During the period of growth analyses were undertaken to determine the blood levels of the estradiol hormone. Samples were harvested for histologic examination. Histological samples were collected differently, depending on the embryonic development and stained HE, HEA, Novelli and van Giesson. The histological samples were examined at the optical microscope motic *B1-211A* camera *Moticam 1000.*

Quails eggs incubation is made under conditions resembling those of hens, being part of the same class. The only difference is the period of embryonic development that lasts 16-17 days.

In the 4th incubation day we identified the development area of gonads and genital tract, which occurs nearby the mesonephros. The separate development of gonads is highlighted from the 5th day of incubation, when we notice the differences in size and shape.

Between day 7 and 10 of incubation the main characteristics of the genitalia development manifest: the right ovary regresses and becomes elongated, while the left one develops normally; the genital tract, both male and female, Wolff and Müller develop simultaneously.

Identification of the gonad as ovary was realized by marking the distinctive areas: cortex and medulla. The cortical area is identified from the 7th day of incubation, as a thickening of the epithelium, representing the first criteria of differentiation.

The cortex is the structure that is used for determining the sexual differentiation, because it is absent in the embryonic testis.

At 9 incubation days we noticed the presence of oogonia in cortex.

The semnificative increase of oocytes takes place between 9-11 days of incubation.

At 13 days of incubation, the primordial follicles begin to form, through the arrangement of granulosa precursor cells around the oocytes.

Folliculogenesis in quail occurs during the embryonic stage and at hatching the ovary presents numerous primordial follicles.

In the first day, histologically we noticed the predominance of primordial follicle and rare primary ones in the ovary. The cortical and medulary areas are separated from each other in day-old chicks. This delimitation is lost during the development of the ovary.

The oviduct in day-old quail chicks is barely visible macroscopically, while histologically we distinguished only pseudostratified ciliated epithelium.

The development of follicles occurs by increase in oocyte size and also in number of layers of granulosa cells.

The folds of oviduct mucosa appear around the age of two weeks. The oviductal segments differentiate anatomically at the age of 31 days. Around 4-5 weeks after hatching, the formation of the tubular glands begins with the invagination of the epithelium.

Estradiol levels in plasma increases starting day 31 after hatching.

Ovary development occurs more rapidly compared to oviductal development and may influence its development by hormone control.

The quail ovary morphology presented structural similarities with the ovary of other bird species: the cortex presents numerous follicles in various stages of development.

Ovarian follicles were classified into primordial, primary, previtelline, vitelline and atretic. In the medulla we noticed numerous blood vessels, nerve fibers and interstitial cells.

This study is important because the morphology and physiological role of the oviduct in fowl is more complex than in mammals.

Oviduct morphology in quail is similar to other bird species: infundibulum presents a mucosa with fine folds and a dispersed distribution of blood vessels; magnum is the longest segment and it's volume is attributed to the increased in secretory surface.

The magnum folds, in some areas, can cover the whole oviduct lumen. These folds represent the main criteria of macroscopic differentiation between the oviduct segments.

The folds of the magnum are the highest compared with other segments.

The isthmus contains glands resembling those of the magnum, but are less dense and contain less secretion.

The uterus mucosa is pigmented and it's brown-red color is due to the numerous blood vessels.

Uterus and vagina are histologically highlighted by their musculosa, histological structure that develops in these segments of the oviduct. Musculosa development is in relation with the functions of these segments: holding and expelling of the egg by contractions.

Researches from Chapter VI, entitled *"Immunohistochemistry exam according to the presence of neurotrophins and their specific receptors in ovary and oviduct of quail "* were realized on a number of 49 female embryos and 12 adult quail *Coturnix coturnix japonica,* from which we sampled the ovaries. The oviduct has been harvested only from adults. 5-7µm sections were obtained. Part of these sections were used for immunohistochemistry, marking a single antibody; the other part has been used for the immunofluorescence exam, marking two antibodies. A confocal exam has been on the adult ovary. The Envision system was used in the immunohistochemistry technique. Morphological interpretation was realized with the NikonEclipse *90i* microscope. Images were obtained with the NIS-Elements 4.20 software.

The positivity of neurotrophins and their specific receptors showed differences in intensity depending on follicular stages and oviduct segments.

In adult ovary, the presence of neurotrophins and their specific receptors has been associated with ovogenesis and folliculogenesis. In embryonic ovary, neurotrophins positivity may show their involvement in oocyte survival and also a signal regarding the initiation of primordial follicles.

It can be stated that no factor can act independent and so there are different grades of interaction between neurotrophins and factors within the ovary for the control of folliculogenesis.

The presence of neurotrophins and specific receptors in the reproductive system suggest their involvement in the oviduct secretion and transport functions. The epithelium showed intense positivity of cells for neurotrophins, creating an alternation between positive and negative cells.

Furthermore, the presence of NGF in tubular glands that secrete egg compounds suggests the possibility that they are secreted by these glands and subsequently will be part of the egg.

The role of neurotrophins in reproductive tissue remains speculative, information being limited in terms of the effect and mechanism during the follicular development and growth.

Chapter VII contains final conclusions which summarize the results of the researches.