

Morphogenesis stages of the embryonic development of the Levantine viper (*Macrovipera lebetina obtusa* Dwigubsky, 1832)

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Abstract

The article provides information on the embryonic development of the Levantine viper (*Macrovipera lebetina obtusa* Dwigubsky, 1832), its stages of morphogenesis, and the morphological variability of embryogenesis at the time of ovulation. It was determined that on the day of egg laying, the blood vessels of the provisional organs (allantois and yolk sac) were formed and covered 50-60% of the body surface of the embryo, and the embryos are already in the initial stages of embryonic development morphogenesis (spiral twist of the body and the beginning of the formation of the tongue). It was determined that the Levantine viper has already finished the 33-35 day development period (embryonic period) in the oviducts by the time of ovulation. After the egg was laid outside, the morphogenesis stages of embryonic development during the natural incubation period were determined according to the morphology of the embryos and the level of development of provisional organs, and 7 stages were described. As a result of the morphological study, 4 periods were distinguished in the embryonic development of the Levantine viper: embryo, pre-fetus and fetus (morphogenesis), and hatching periods. The article describes the morphology and development process of embryos in the stages of morphogenesis and embryonic development.

Keywords: Levantine viper, egg, embryo, allantois, yolk sac, embryonic development, developmental stag

Introduction

Although there are many materials dedicated to the embryonic development and morphogenesis of reptiles in the scientific literature, most of them only provide information on the embryogenesis of individual definitive organs. C. Pasteels (1957) morphologically described the process of gastrulation and several developmental stages in the embryonic development of the turtles and lizards. Zehr David (1962) studied and described the stages of embryonic development in the snakes *Thamnophis sirtalis sirtalis* in the period before and after oviposition. Kochva E. (1963) studied the development of the venom gland and trigeminal muscles in *Vipera palaestinae*. J Dafaure and J Hubert (1961, 1966) identified 40 stages of development of viviparous lizard embryos (*Lacerta vivipara*), described gastrulation, early stages of development of the amnion and allantois. In the future these authors noted 13 stages in the embryonic development of field viper *Vipera aspis* based on the development levels of provisor organs. Danielyan F.L., Simonyan A.A. (1976) studied the embryonic development of the sand lizard (*Lacerta agilis*) and identified 13 stages. The scientific literature notes that in snakes that reproduce by laying eggs, the first period of embryonic development ends in the oviducts before the eggs are laid outside. L.G. Korneva (1969, 1976) studied the stages of the embryonic development of the watersnake *Natrix tessellata* and determined that the eggs are laid outside at the 30-33 day stage of embryo development, after the end of the embryonic period. Research is continued today to study the embryonic development of snakes [Marcela Buchtova et al., 2007; Anthony et al., 2012; Khannoon et al., 2014; Maria Tereza et al., 2020]. Our presented article is devoted to the study of the morphogenesis stages of embryonic development during artificial incubation of Levantine viper eggs.

Materials and methods

The research materials were embryos obtained by artificial incubation of freshly laid eggs of the Levantine viper (*Macrovipera lebetina obtusa* Dwigubsky, 1832). The materials were collected in 1976-1977 at the Snake Venom Producing Herpetological Combine and in 2015-2017 at the experience base of the ANAS Zoological Institute in Aghsu region. For this purpose, during the reproduction season (June-July), 442 hatchable eggs obtained from 48 "pregnant" vipers brought from the wild and kept in a cage or aviary were artificially incubated (Fig.1).



Figure 1. Eggs of the Levantine viper (*Macrovipera lebetina obtusa* Dw.,1832)

Eggs were incubated in special plastic containers in a sterilized substrate (sawdust or dried moss) under special temperature (29-31 C) and relative humidity (70-80%) conditions [Искендеров, 1978]. During the study, the stages of morphogenesis of the embryonic development of the viper, and morphological changes of embryogenesis on the day of ovulation were studied. On the day of ovulation, 1 egg was removed from each egg ball the morphology of the embryo and the level of development of provisional organs (amnion and allantois) were studied and the stage of morphogenesis was determined. In the later period of incubation, 2-3 eggs were removed every 3-5 days and the morphogenesis stage of the embryo was described. Embryos of female species belonging to different populations were morphologically compared to study the morphological variability of embryogenesis on the day of egg-laying in populations settled in lowland and mountainous areas.

Results and discussion

It is known that the embryonic development of snakes that reproduce by laying eggs, including the Levantine viper, includes 4 periods: embryo, pre-fetus and fetus (morphogenesis), hatching periods [Корнева, 1976; Искендеров, 1978]. It is noted that the embryonic period ends when the eggs are in the oviducts of the female. The stages of embryonic development during the incubation period after the eggs were laid outside were determined by taking into account the morphological characteristics of the embryos, as well as the levels of development of provisional organs:

1. *The stage of formation of the spiral twist of the body and the base of the tongue* (Egg-laying day) (Fig. 2,-1). At this stage, the beginning of all provisional (allantois and yolk sac) and definitive organs is established. The contour of the embryo resembles a spiral twisted 4.0-4.5 times. The head is in a tilted position of 45° relative to the body. The lower jaw is about half as long as the upper jaw. Under the skin epithelium, the beginning of the pituitary gland, the spinal cord, muscle segments and blood vessels can be seen. The beginning of the hemipenile sacs around the cloaca is felt in the form of bulging. These sac derivatives in the form of blisters remain outside until a certain stage of development. The side walls of the body are formed to the level of the heart. The sclera of the eye is densely pigmented. The nasal cavity is closed. On the lower edges of the upper jaw, the beginning of the poison teeth is felt. At the bottom of the oral cavity, the beginning of the tongue appears in the form of a bulging. Provisional organs already cover 50-60% of the body surface of the embryo. The embryo is in the amniotic sac, which is filled with amniotic fluid and is deposited in the yolk sac. More than half of the yolk sac is covered with a network of blood vessels, but the edges are not yet united. The allantois has not yet fully covered the embryo. In its network of blood vessels, the vessels are larger and less branched. Respiration of the embryo is provided through allantois and yolk sacs. The edges of the yolk sac are not attached to the embryo, and it is left open in the form of a "window". Through this "window" the feeding umbilical cord connects to the embryo. The size of the "window" is 3 x 7 mm. At this stage, the wet weight of the embryo is 0.24 g, the length is 63 ± 3 mm, and the width of the head is 4 ± 1 mm.

1. *The stage of fusion of the edges of provisional organs* (6th day of incubation) (Fig 2,-2). By this time, the embryo has already increased in mass compared to the previous stage and reaches the pre-embryonic stage, it sinks deeper into the yolk, keeping the shape of a spiral twist. The front part of the spiral twist is slightly opened and released, and the embryo consists

of 3 rings. At the level below the heart, the body walls are formed, and the lumbar scales begin to be felt. The heart bulging is reduced, the lower jaw is about 2/3 of the length

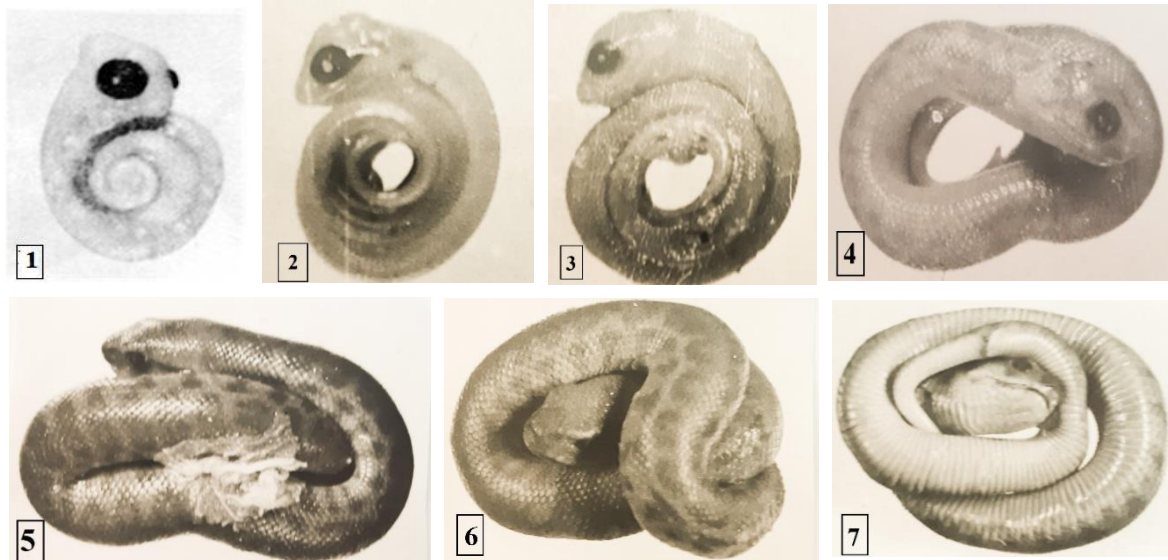


Figure 2. Stages of embryonic development of the Levantine viper (*Macrovipera lebetina obtusa* Dw.,1832):

of the upper jaw. Provisional organs in the edges of the yolk sac of the body have fused and reached their maximum size. The edges of the allantois are not completely united, but remain open in the form of a small hole. As the embryo sinks deeper into the yolk, its open "window" becomes narrower and its size is 5 x 3 mm. At this stage, the wet mass of the embryo is 0.86 g, the length is 78 ± 13 mm, the width of the head is 5 mm, and the width of the body is 4 mm.

2. *Reaching the maximum length of the lower jaw (12th day of incubation)* (Fig. 2,-3).

The opening of the spiral twist of the embryo continues, the last ring is hardened, and the body spiral already consists of 2.5 rings. The edges of the side walls of the body are fused above the heart, and fully formed below the heart to the anus, but the edges are not yet fused. The length of the lower jaw has reached the tip of the upper jaw. The tip of the spade-shaped wide tongue is crossed. At the base of the tongue is the beginning of the vagina of the tongue in the form of a small bulging. At the bottom of the yolk sac, the edges of the allantois are completely united and it takes over the respiratory function of the embryo. The yolk sac performs only a nutritional function. The body is completely covered with abdominal scales in the ventral part. As the mass of the embryo increases in the pre-fetus stage, it sinks deeper into the yolk substance. The size of the "window" of the yolk sac is 6 x 4.5 mm. The mass of the embryo is 1.6 g, the length is 100 ± 16 mm, the width of the head is 5 mm, the width of the body is 4.5 mm.

3. *Beginning of body pigmentation (18th day of incubation)* (Fig. 2.-4). At this stage, the embryo in the fetal period loses its spiral twist shape and consists of 2 free rings. Horning of the dorsal scales of the body begins and weak pigmentation is observed in the scales. The edges of the side walls of the body are joined throughout the body. The skin epithelium of the head is slightly thickened. Eyelids are formed. The curvature of the head has disappeared and it is located along a plane with the body. Shields are formed on the head. The edges of the nostrils are joined. Internal organs are visible from the side walls of the body, including 2 long white tapes of fat extending from the liver to the umbilical protrusion. These tapes are of the same length and are parallel. The mass of the embryo is 3.04 g, the length is 125 ± 23 mm, the width of the head is 7 mm, the width of the body is 6 mm.

4. *Formation of head and body patterns (28th day of incubation)* (Fig. 2.-5). Body pigmentation has intensified. The body has a checkerboard pattern characteristic of the species. The head and tail areas are pigmented. Pigmentation also spreads to the sides of the body. The scales on the head are fully formed. Venom fangs are still made of cartilage. The tongue is thinned and elongated. Abdominal walls are still thin and transparent, internal organs are visible. The mass of the internal organs located in the form of a tape constitutes 2.1% of the total body mass. The mass of the embryo is 5.2 g, the length is 145 ± 30 mm, the width of the head is 8 mm, the width of the body is 7 mm. The size of the yolk sac is still small (9 x 10 mm).

5. *Pulling the yolk sac over the embryo (fetus) (35th day of incubation)* (Fig. 2.-6). There were no serious changes in the appearance of the fetus. Pigmentation and dimensional elongation of the fetus increases. Fat continues to accumulate in the body cavity. The mass of fat is 0.27 g, which makes up 3.4% of the total mass of the fetus. The formation of the abdominal walls and its covering with scales has been completed. Under the chin and throat, as well as the tail areas, are covered with scales. Nostrils are closed. In the yolk sac, the yolk substance has decreased and is in granular form. The yolk sac is nearing completion of complete descent over the fetus and the "window size is maximum (18 x 28.5 mm). The mass of the fetus is 7.9 g, the length is 145 ± 37 mm, the width of the head is 9 mm, and the width of the body is 8 mm.

6. *The complete sliding of the yolk sac over the fetus, absorption of the remaining provisional organs into the body cavity of the fetus (40-41st day of incubation)* (Fig. 2.-7). The yolk sac completely slides over the body of the fetus. The fetus is connected to the yolk only through the umbilical cord. Respiration ends with the allantois. At this time, the suffocation of the fetus causes it to move violently, and the eggshell is cut with the sharp tip of its face, the fetus comes out of the amniotic sac and pushes its head out of the egg. From this moment,

pulmonary respiration starts. Although the function of the allantois and the amnion ends, the yolk sac still maintains its function and nourishes the fetus. The remains of the provisional organs begin to be absorbed into the stomach of the newly hatched snake. At this time, the young snake circulates the rest of the temporary organs with its own body. It may take 8-12 hours for the remains to be completely absorbed into the body cavity of the young snake. The remains absorbed into the body cavity are enough for the baby snake to live without food for a while. At this stage, the only change that occurs in the external appearance of the fetus is the transition of the hemipenis to the inside of the body. The embryo does not differ from the adult individuals in appearance, the poison teeth are completely ossified. Finally, the annular body of the fetus opens completely and leaves the egg. The weight of the hatched snake is 9.8 g, its length is 176 ± 31 mm, the width of the head is 10.5 mm, and the width of the body is 10 mm. Thus, during the artificial incubation period were described, according to the morphology of the embryos and the level of development of the provisional organs 7 stages of the embryonic development of the Levantine viper. As a result of the morphological study, 4 periods were distinguished in the embryonic development of the Levant viper: embryonic, pre-fetal and fetal (morphogenesis), hatching periods.

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