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Study of embryos stages of development for estimation of day of death in red-legged partridge (*Alectoris rufa rufa* L.)

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The exact determination of the death age of embryos could be important in determining causes of embryonic mortality. For the lack of better references, technicians refer to pheasant or chicken embryo development in order to analyse unhatched partridge eggs. For this reason, a study on partridge chick embryo development was useful.

To monitor red-legged partridge (*Alectoris rufa rufa*) embryo development, we incubated 80 eggs, chosen randomly, all laid in the same day of the 9th laying week. The eggs' longitudinal and transversal diameters were of 38.0±6.4mm (mean±SD) and 30.2±1.0mm, respectively. Egg weight averaged 19.2±1.4g. Incubation was at a temperature of 99.7°F (37.61°C) and a humidity of 47%RH. During hatching temperature was 99°F (37.2 C) and relative humidity was 47%. Room temperature and humidity were 75.2°F (24°C) and 55% RH. Every day during the incubation period 2 eggs were opened, embryos were photographed, described in a macroscopic manner and the following dimensions were measured: longitudinal and transversal egg diameters, egg weights, maximal length amnion diameter, maximal length side embryos (in natural position), maximal length embryos (expanded), eyeball diameter, length of whole beak structure, length of the external beak portion (opening side), and the length of the humerus, the carpal and metacarpal, the femur, the tarsal and metatarsal and the 3rd toe. All measurements were made with callipers and the mean values are given. All embryos were stored in a 40% formaldehyde solution.

In order to estimate embryo age, we can divide the whole development process into two main periods. The first period (indicated by blue sections in Table 1) is mainly characterised by formation of new organs (embryonic or extra embryonic or body portions), going from the first to 17th incubation day. The second period (indicated by yellow sections in Table 1) is characterised by growth of body organs and limbs from the 18th day to the 24th.

The study also elucidated enough development stages to estimate embryo age within an approximation of about one day. Particularly, as briefly shown in Table 1, the study showed that on the 3rd day, the area vasculosa ring is completed and reaches a diameter of 16 mm. Also, cardiac activity begins on the 3rd day. By the 4th day, eyes primary formations appear and, on the 5th day start their pigmentation; furthermore, on the 5th the wing buds appear; hind limb buds appear on the 6th day; the beak primary formation appears on the 8th day; the scleral papillae, the egg tooth and the eyelids appear on the 9th day; on the 10th day the feather germs are visible; on the 11th day a few black feathers start to form and the uropygial gland becomes visible. On the 15th day, the claw buds are distinguishable.

While in previous days the femur length increased steadily, by the 18th day, it is about 13 mm and only increases to 14 mm by the 20th day. Therefore, at least another measurement is required in order to improve the accuracy of estimation. The 3rd toe is 12.5 mm on the 18th day, 14.5 mm by the 19th day and 16 mm by the 20th day. On day 23, the yolk sac is still not completely drawn into the body, but all the extra embryonic membranes appear dry and degenerating because blood circulation has stopped, with only remnants present. At the same time, the beak embryo is already in the air chamber and lung respiration has begun. Finally, on day 24, the yolk sac is completely drawn into the body and the chick hatches.

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Table 1. Timetable of partridge embryo development.

Day	Main Topic	Note	Picture	Day	Main topic	Note	Picture
0	Blastoderm	Before starting incubation: white spot on the top of the yolk; diameter 4 mm. <i>Area pellucida</i> diameter 2 mm		13	Plantar cushion; ears	Plantar cushion bumps appear; acoustic meatus well visible; feather on head and wings	
1	Blastoderm	Blastoderm diameter 7 mm; area pellucida diameter 2.6 mm		14	Yolk sac and allantois membrane	Yolk sac closed; allantois completely adhere to the shell membranes	
2	Blastoderm	Blastoderm diameter 21 mm; area vascolosa "U" shaped		15	Claw	Claw primary formation are visible	
3	Area vascolosa; embryo	Circle ring diameter 16.3 mm, 2 main vessels branch; cardiac activity visible; head, eyes and spine sketch visible		16	Plantar cushion and claw, feet skin pigmentation	Plantar cushion completely formed; claws almost formed and easily recognisable; pigmentation of feet on the upper side	
4	Eyes	Eyes well defined		17	Eyelid and femur	Eyelid primary pigmentation and covering the whole eyeball; femur length 11.4 mm; 3 rd toe 12 mm	
5	Eyes and wings	Eyes pigmentation and wing buds appearance		18	Femur and 3 rd toe	Femur length 13 mm, 3 rd toe length mm 12.5	
6	Leg sketch	Leg buds distinguishable		19	Femur and 3 rd toe	Femur length 13.5, 3 rd toe length mm 14.5	
7	Choroid fissure	Eye pigmentation darkened contrasting with choroids fissure		20	Femur and 3 rd toe	Femur length 14 mm, 3 rd toe length 16 mm	
8	Beck sketch and scleral papillae	Beck and scleral papillae sketches appearance		21	Femur and 3 rd toe	Femur length 15 mm, 3 rd toe length 17 mm	
9	Scleral papillae, egg tooth and eyelids	Scleral papillae complete all circle, egg tooth appears as a mobile white little ball and edges of eyelids become visible		22	Femur and 3 rd toe	Femur length 15.5 mm, 3 rd toe length 18 mm	
10	Feather germs	Feather germs appearance		23	Yolk sac and extra embryonic membranes	Yolk sac almost drawn into the body and extra-embryonic membranes degenerating. Extra-embryonic blood circulation stopped and lung respiration started	
11	Black feather sketch	Black feathers primary formation appearance		24	Yolk sac	Yolk sac completely drawn into the body and spontaneous hatching	
12	Eyelids and ears	Eyelids cover 2/3 of the entire eyeball and nictating membrane well formed; just visible acoustic meatus					