EMBRYO STAGES OF DEVELOPMENT FOR ESTIMATION OF DAY OF DEATH IN PHEASANT (Phasianus colchicus) AND PARTRIDGES (Alectoris rufa AND Perdix perdix)

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ABSTRACT

In captivity condition the rearing of many game bird species is affected by a low hatching rate. Also, there are insufficient specific references about the embryo development of these species. The exact estimation of the embryos’ death age is the basis for carrying out the diagnosis of the causes of death during incubation and, consequently, for improve hatchability.

For these reasons a comparative study on embryo development was carried out on pheasant (Phasianus colchicus), red-legged partridge (Alectoris rufa) and grey partridge (Perdix perdix). For each species, 100 eggs laid in the same day of the 9th laying week were incubated contemporarily. At least 3 eggs for each species were opened daily during the incubation. Embryos were photographed, described in a macroscopic manner and the main measurements of anatomic portions were taken.

The day of appearance of new organs and body portion (e.g. eye, claw, beck, etc.) was determined and the study allowed sufficient elucidation of the development stages to estimate macroscopically embryo age within an approximation of about one day. A general delay in the embryo development and hatching time of the grey partridge was observed in comparison to pheasant and red-legged partridge.

INTRODUCTION

Game bird rearing is still affected by bad reproduction performances in comparison to other avian species, usually intensively reared for human food production. The low egg hatchability is one of the parameters affecting the reproduction success of game birds. In fact, while in chickens average hatchability is 85-88%, in game bird species it is 65-80 % (Bagliacca, 1996; Gonzalez Redondó, 2006). The exact determination of the age of death can be useful in determining the embryonic mortality causes, so that technicians and farmers can act on the rearing process and/or on the incubation settings, to improve the hatching results of game birds. Actually, technicians and farmers refer mainly to the chicken or the pheasant embryo-development, in order to examine the unhatched eggs. The aim of the present study, was to set up an “instrument” that could be useful for veterinarians,
technicians but also farmers, to establish the exact day of death also in red-legged and grey partridges, and comparing their development to that of the pheasant. Only macroscopic determinations, very simple, immediate and available to all technicians and farmers, were used to describe the embryo development of these species.

MATERIALS AND METHODS
To monitor embryo development, 100 eggs were incubated for each considered species. The eggs, laid in the same week, were incubated in the same machine. The incubator was set at a temperature of 99.7 °F (37.6°C) and at a relative humidity of 47 % (82 °F wet bulb). The hatcher was set at a temperature of 99.0 °F (37.2 °C) while relative humidity was 47 % (38 °F wet bulb) before pipping and 56 % (86 °F wet bulb) during the hatch. Temperature and humidity of the room where the machine was located, were 75.2 °F (24 °C) and 55 %, respectively. Every day during the incubation period 3 eggs were opened, embryos were photographed, macroscopically described and the following measurements were recorded: longitudinal and transversal egg diameters and weight, amnion diameter, embryo length (stretched out), eyeball diameter, length of beak, length of the whole limbs, of humerus, carpal and metacarpal, femur, tarsal and metatarsal and 3rd toe. All measurements were made with callipers their mean values were recorded.

RESULTS
The used egg’s characteristics are reported in table 1. The whole development process of the embryos was subjectively divided into two main periods, as already described in a previous study carried out only on red-legged partridges (Fronte et al., 2006). A first period, mainly characterised by formation and development of new organs, went from the first to 17th incubation day. A second period, characterised by the growth of some body organs or portion (limbs or their portions mainly), went from the 18th day to the hatch.

<table>
<thead>
<tr>
<th>Species</th>
<th>egg weight</th>
<th>egg trasv. T</th>
<th>egg long. T</th>
<th>E. shell + mem thickness</th>
<th>Egg shell</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. colchicus</td>
<td>29.8±2.59 A</td>
<td>34.9±1.03 A</td>
<td>44.4±2.30 A</td>
<td>30.8±4.07 A</td>
<td>26.8±2.41 A</td>
</tr>
<tr>
<td>A. rufa rufa</td>
<td>19.1±1.47 B</td>
<td>29.8±1.31 B</td>
<td>38.6±2.59 B</td>
<td>29.9±3.68 A</td>
<td>25.1±3.05 B</td>
</tr>
<tr>
<td>P. perdix</td>
<td>14.0±0.79 C</td>
<td>26.8±0.61 C</td>
<td>35.5±1.21 C</td>
<td>26.9±3.80 B</td>
<td>22.5±3.90 C</td>
</tr>
</tbody>
</table>

Note: means with different letters differ per p<0.01.

The whole embryo development stages are summarized on Table 2. The three species were very similar in their development. Particularly, on the 3rd day, the area vasculosa ring is completed and reaches a diameter of about 17.3 mm in pheasant, 13.9 mm in red-legged partridge and 9.7 mm in grey partridge. By the 4th day, eye primary formations appear in every species and on the 5th day eye pigmentation
starts; furthermore, on the 5th the wing buds appear while hind limb buds appear on the 6th day; the beak primary formation appears on the 8th day; the egg tooth and the eyelids appear on the 9th day; on the 10th day the feather germs are visible.

Table 2

<table>
<thead>
<tr>
<th>Pe</th>
<th>Ar</th>
<th>Pp</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. colchicus</td>
<td>A. rufa rufa</td>
<td>P. perdix</td>
</tr>
</tbody>
</table>

Day 0: (before incubation); BL on the YK surface, 0.5±1.28 mm; translucent area inside the white and dull area 2.8±0.73 mm Pe→Ar→Pp.

Day 1: BL on the YK surface, 0.6±1.61 mm; translucent area inside the white and dull area 2.7±0.61 mm Ar→Pe→Pp.

Day 2: BL on the YK surface, 18±6.77 mm Pe→Pp→Ar; U shaped AV always present on Ar, sometimes on Pe and Pp, possible presence of the primitive streak, only for PH, always invisible for RP and GP.

Day 3: presence of a well visible vascularised area. Vascular ring at Pe (17.3±6.68a), Ar (13.9±2.65a) Pp (9.7±0.85b); Pe and Pp with a bordering vascular ring.

Day 4: E longer in Pe (more than 2.5 mm) than in Ar and Pp; EV: very well defined only in Pe, recognisable in Pp and just recognisable in Ar; EV: dark spot on the Pp E head only.

Day 5: EV well pigmented in Pe and Ar, very few in Pp; A1 present in Pe and Ar but not always in Pp. E migrated to the wide egg-side in Pe, E I well recognisable only in Pe and Ar.

Day 6: E I. Ar (10.8) > Pe (14.5) > Pp (14.5); amniotic vesicle at Ar (13.5) > Pe (12.5) > Pp (12.0), E I - wing, Ar (2.96) > Pe (2.5) > Pp (0.8), leg Ar (3.1) > Pe (2.0) > Pp (1.8); EV: pigmented; VM appears thicker, more vascularised and translucent in Pe and Ar than in Pp.

Day 7: E I. Pe → Ar (23) > Pp (20); amniotic vesicle at Ar (14.9) > Pe (19.6) > Pp (16.5); wing Ar (4.0) > Pe (3.6) > Pp (2.8); leg Ar (4.9) > Pe (4.7) > Pp (3.5); EV: Ar (4.0) > Pe (4.1) > Pp (3.1).

Day 8: BK well developed in Pe and Ar, not completely formed in Pp; knee articulation always recognisable in Ar, sometimes in Pe, not yet visible in Pp; EV: dark in Pe and Ar while appears almost grey in Pp; E, pink coloured in Pe and Ar, still translucent in Pp.

Day 9: egg tooth appears in every species as white dot on the upper BK. PY at Pe (6.5) > Ar (6.0) > Pp (5.0); Pe→Pp; eyelid still translucent and slit (distance between the two opposite edges) Pe (6.5) > Ar (4.5) > Pp (3.5); E I: Pe (27.0) > Ar (20.0) > Pp (23.9).

Day 10: A M becomes invisible, BK becomes quite rigid and the main structures visible as dull tissues; FG easily recognisable in Pe, with difficulty in Ar, not discernible in Pp; E I: Pe (10.3) > Ar (28.8) > Pp (27.0); eyelids slit: Pe (5.1) > Ar (4.5) > Pp (4.1).

Day 11: 3rd eyelid appears in every species. FG macroscopically discernible in Pp and well visible on the outer side of the leg in Ar and on the breast and along the spine in every species.

Day 12: FG diffused all over the body; FT present along the spine, on the outer side of the leg and on the tail in Ar, only along the spine in Pe and not yet present at all in Pp; E I: Pe (2.2) > Ar (1.6) > Pp (0.9); (Pe→Pp).

Day 13: FT appears in GP too, but still less diffused than in the other species; two colours can be seen in FT of every species.
on the 11th day a few black feathers start to form. On the 15th day, the claw buds are distinguishable. After this age, when determination of the exact day of death is required, it is necessary to add also some embryo measurements to the macroscopic observations. The best measures are the femur and the humerus lengths and, starting from the 20th day, the length of the 3rd toe too. On day 23, the yolk sac is still not completely drawn into the body, but the extra embryonic membranes appear dry and degenerating because blood circulation has stopped. At the same time, the beak embryo is already in the air chamber and lung respiration begins in pheasant and red-legged, not yet in grey partridge. Finally, on day 24, the yolk sac is completely
drawn into the body and the chicks hatch in pheasant and red-legged partridge but not always in grey partridge.

**DISCUSSION**

The study elucidated the main evident daily development stages of the embryos and thus allows us to macroscopically estimate death-embryo ages in the unhatched eggs with an approximation of about one day.

Regarding relevant differences between species, a general slower embryo development was observed in grey partridge in comparison to the other species. This development delay can be linked to the “maturation” of the embryo, for example, to the strength of the embryo tissues, their thickness and their colours (generally lighter and more translucent) but also to the inclusion process of the yolk sac into the abdomen. For these reasons, a longer incubation period of at least 12 hours is necessary to allow grey partridge chicks to hatch.

**REFERENCES**


