1ST Mediterranean Summit of WPSA

Advances and Challenges in Poultry Science

BREEDING AND REPRODUCTION

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

B. Fronte[1]*, E. Cacciuttolo[2], F. Giuliacci[1], P. Mani[2], M. Bagliacca[1]

Book of Proceedings

Editors:
A. Tserveni-Goussi
A. Yannakopoulos
P. Fortomaris
G. Arsenos
E. Sossidou

07 – 10 MAY 2008
Porto Carras, Chalkidiki

EDUCATION ORGANIZATION RESEARCH
Greek Branch

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

B. Fronte[1]*, E. Cacciuttolo[2], F. Giuliacci[1], P. Mani[2], M. Bagliacca[1]

[1] Department of Animal Production
[2] Department of Animal Pathology
Veterinary College, University of Pisa, Viale delle Piagge 2, 56124 Pisa, Italy.
bfronte@vet.unipi.it

Keywords: game birds, embryo development, pheasant, partridge, hatchability

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 Redondo, 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 øF (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 1. CHARACTERISTICS OF THE EGGS OF THE DIFFERENT SPECIES

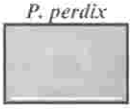
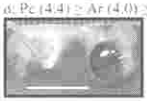
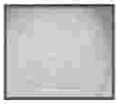
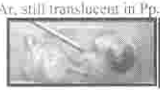
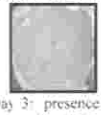
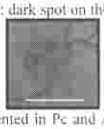
Species	egg weight	egg trasv. T	egg long. T	E. shell + mem thickness	Egg shell
P. colchicus	29.8±2.59 A	34.9±1.03 A	44.4±2.30 A	30.8±4.07 A	26.8±2.41 A
A. rufa rufa	19.1±1.47 B	29.8±1.31 B	38.6±2.59 B	29.9±3.68 A	25.1±3.05 B
P. perdix	14.0±0.79 C	26.8±0.61 C	35.5±1.21 C	26.9±3.80 B	22.5±3.90 C

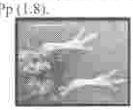
Note: means with different letters differ per $p<0.01$.

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

Pc *P. colchicus*	Ar *A. rufa rufa*	Pp *P. perdix*	Pc *P. colchicus*	Ar *A. rufa rufa*	Pp *P. perdix*
					
Day 0 (before incubation): BL on the YK surface, ø 5.5±1.28 mm; translucem area inside the white and dull area 2.9±0.73 mm Pp>Pc≥Ar.			Day 7: E l: Pc – Ar (21) ≥ Pp (20); amniotic vesicle ø: Ar (19.3) ≥ Pc (19.0) ≥ Pp (16.5); LI l: wing Ar (4.0) ≥ Pc (3.6) ≥ Pp (2.8); leg Ar (4.9) ≥ Pc (4.7) > Pp (3.5); EY ø: Pc (4.4) ≥ Ar (4.0) > Pp (3.3).		
					
Day 1: BL on the YK surface, ø 6.5±1.61mm; translucent area inside the white and dull area 2.7±0.61 mm Ar≥Pc≥Pp			Day 8: BK well developed in Pc and Ar, not completely formed in Pp; knee articulation always recognisable in Ar, sometimes in Pc, not yet visible in Pp; EY: dark in Pc and Ar while appears almost gray in Pp, F: pink coloured in Pc and Ar, still translucent in Pp.		
					
Day 2: BL on the YK surface, ø 18±6.7mm Pp≥Pc≥Ar; U shaped AV always present on Ar, sometime on Pc and Pp; possible presence of the primitive streak only for PH, always invisible for RP and GP			Day 9: egg tooth appears in every species as white dot on the upper BK; EY ø: Pc (6.5) ≥ Ar (6.0) ≥ Pp (5.0) (Pc>Pp); eyelid still translucent and slit (distance between the two opposite edges) Pc (6.5) ≥ Ar (4.5) > Pp (3.5); E l: Pc (29.2) ≥ Ar (26.3) ≥ Pp (23.9).		
					
Day 3: presence of a well visible vascularised area. Vascular ring ø: Pc (17.3±6.65a), Ar (13.9±2.95a) Pp (9.7±0.58b); Pc and Pp with a bordering vascular ring.			Day 10: AM becomes visible; BK becomes quite rigid and the main structures visible as dull tissues; FG easily recognisable in Pc, with difficulty in Ar, not discernible in Pp, F l: Pc (30.3) ≥ Ar (28.8) ≥ Pp (27.0); eyelids slit: Pc (5.1) ≥ Ar (4.5) ≥ Pp (4.1).		
					
Day 4: E longer in Pc (more than 2.5 mm) than in Ar and Pp; EV: very well defined only in Pc, recognisable in Pp and just recognisable in Ar; EY: dark spot on the Pp E head only.			Day 11: 3rd eyelid appears in every species; FG macroscopically discernible in Pp and well visible in the outer side of the leg in Ar and on the breast and along the spine in every species.		
					
Day 5: EY well pigmented in Pc and Ar, very few in Pp; AL present in Pc and Ar but not always in Pp; E migrated to the wide egg-side in Pc; LI well recognisable only in Pc and Ar			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 Pc and not yet present at all in Pp, E wl: Pc (2.2) ≥ Ar (1.6) ≥ Pp (0.9) (Pc>Pp).		
					
Day 6: E l: Ar (16.8) ≥ Pc (14.5) ≥ Pp (14.5); amniotic vesicle ø Ar (13.5) ≥ Pp (12.5) ≥ Pc (12.0); LI l: wing Ar (2.9) ≥ Pc (2.5) ≥ Pp (0.8), leg Ar (3.1) > Pc (2.0) ≥ Pp (1.8); EY: pigmented; VM appears thicker, more vascularised and translucent in Pc and Ar than in 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.		

Pc	Ar	Pp	Pc	Ar	Pp
P. colchicus	*A. rufa rufa*	*P. perdix*	*P. colchicus*	*A. rufa rufa*	*P. perdix*
					
Day 14: feather ring surrounding AM in Ar and Pp but not in Pc; nose meatus can be seen on the upper BK in every species; eyelids not completely closed, Pc average slit about 2 mm, Ar and Pp 1÷1.5 mm, no slit in one Ar; PC bumps and claws are recognisable. E wt: Pc (3.5) > Ar (2.7) > Pp (1.8).			Day 20: E wt Pc (10.3) > Ar (8.0) > Pp (5.2); humerus: Pc (13.0) ≥ Ar (12.0) ≥ Pp (10.0); femur: Pc (16.8) > Ar (14.3) ≥ Pp (12.5); 3rd toe (claw included): Pc (16.0) > Ar (15.0) > Pp (11.0); FT: plumes entirely cover the eyelids; nose meatus edge entirely darkly pigmented in Pc, only in part in Ar and not pigmented at all in Pp.		
					
Day 15: CL visible as white buds; PC visible but not completely formed; AM surrounded by feathers only along the anterior edge in Pc, by a complete feather ring in Ar and Pp; Eyelids closing eyeball only in some subject of every species; E wt: Pc (4.4) > Ar (3.4) > Pp (2.3); FT appear on several new body areas.			Day 21: E wt: Pc (11.6) > Ar (8.6) > Pp (5.6); humerus: Pc (14.0) ≥ Ar (12.5) > Pp (10.0); femur: Pc (18.0) ≥ Ar (16.5) > Pp (13.5), 3rd toe (claw included): Pc (17.0) ≥ Ar (15.0) ≥ Pp (12.8) (Pc>Pp); nose meatus not yet pigmented in Pp.		
					
Day 16: E wt: Pc (6.1) > Ar (3.7) ≥ Pp (2.5); eyelids closing the eyeball in every bird with FT surrounding eyelids; BK): Pc (7.9) ≥ Ar (7.0) ≥ Pp (6.8).			Day 22: E in hatching position (with head under right wing) in Pc, not in every Ar and Pp.		
					
Day 17: E wt: Pc (7.0) > Ar (4.9) > Pp (3.1); humerus: Pc (10.8) ≥ Ar (9.5) ≥ Pp (8.5) (Pc>Pp); femur: Pc (13.0) ≥ Ar (11.3) ≥ Pp (10.0) (Pc>Pp); FT cover quite all the dorsal portion of the body; slough appears on feet and leg skin of Pc and Ar.			Day 23: Every E in hatching position, BK already in the air chamber in Pc and Ar but not yet in Pp; lung respiration (lung red coloured) already started in Ar but not yet in Pc; YK not completely included into the abdomen of every E.		
					
Day 18: E wt Pc (8.1) > Ar (4.8) ≥ Pp (3.7); humerus: Pc (12.1) > Ar (9,8) ≥ Pp (9.0); femur: Pc (14.0) > Ar (11.7) ≥ Pp (11.0); eyelids edges pigmentation starts in PH only; toe skin pigmentation starts in Ar only; VM covers the whole YK; slough appears on feet and leg distal portion skin of Pp too.			Day 24: Pp hatching occurs many hours later than in Pc and Ar; some Pp embryos have not yet entirely included the yolk sac into the abdomen.		
					
Day 19: E wt Pc (8.6) > Ar (6.0) ≥ Pp (4.4); humerus: Pc (11.8) ≥ Ar (10.3) ≥ Pp (9.8); femur: Pc (14.5) > Ar (14.3) ≥ Pp(12.5), FT: almost complete and plumes appear on eyelids too, pigmentation starts on Ar tarsus and Pc eyelid edges.					

Legend: AL: Allantoic vesicle, AM: Acustic meatus, Ar: Red-legged partridge, AV: Area Vasculosa, BK: Beak, BL: Blastoderm, BV: Blood vessel, E: Embryo, EV: Encephalin vesicles, EY: Eye, FG: Feather germ, FT: Feathers, HR: Heart, l: lenghtness, LI: Limbs, Pc: Pheasant, PC: Plantar cushions, Pp: Grey partridge, th: thickness, VM: Vitellin membrane, w: wideness, wt: weight, YK: Yolk, ø: diameter.
Note: *measurement are given in mm and g; the white line in the pictures correspond to 1 cm; the symbol ">" mean statistical difference for P<0.05, "≥" mean no statistically significant difference.

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

Bagliacca M. (1996). Proc. of the XX World's Poultry Congress, Deli, India: 740-749.

Barasa, A., Dellardi, S., Monge, F., Baroni, E. & Monetti, P.G. (1988). Riv. avicoltura. 57, 73-89.

Fronte B., Cacciuttolo E., Mani P., Bagliacca M. (2006). Avian and Poultry Biol. Rev. 17, (2), 62-63.

Hamburgher, V. & Hamilton, H.L. (1951). J. Morph., 88, 49-67.

Kaltofen, R.S. (1971). Centre for agricultural publishing and documentation. Wageningen. 1-72.

P. González-Redondo (2006) – J. Appl. Poult. Res. 15:579-583

First published: Thessaloniki 2008 **ISBN 978- 960-12-1686-7**

Printed and bound in Greece by the

UNIVERSITY STUDIO PRESS
Publishers of Academic Books and Journals

32, Armenopoulou Str., GR - 54635 THESSALONIKI - GREECE
Tel. +30 2310 208731, +30 2310 209637
Fax +30 2310 216647 • e-mail: univstud@spark.net.gr

STOA TOU BIBLIOU – 5, Pesmazoglou Str., GR - 10564 ATHENS
Tel. & Fax +30 210 3211097