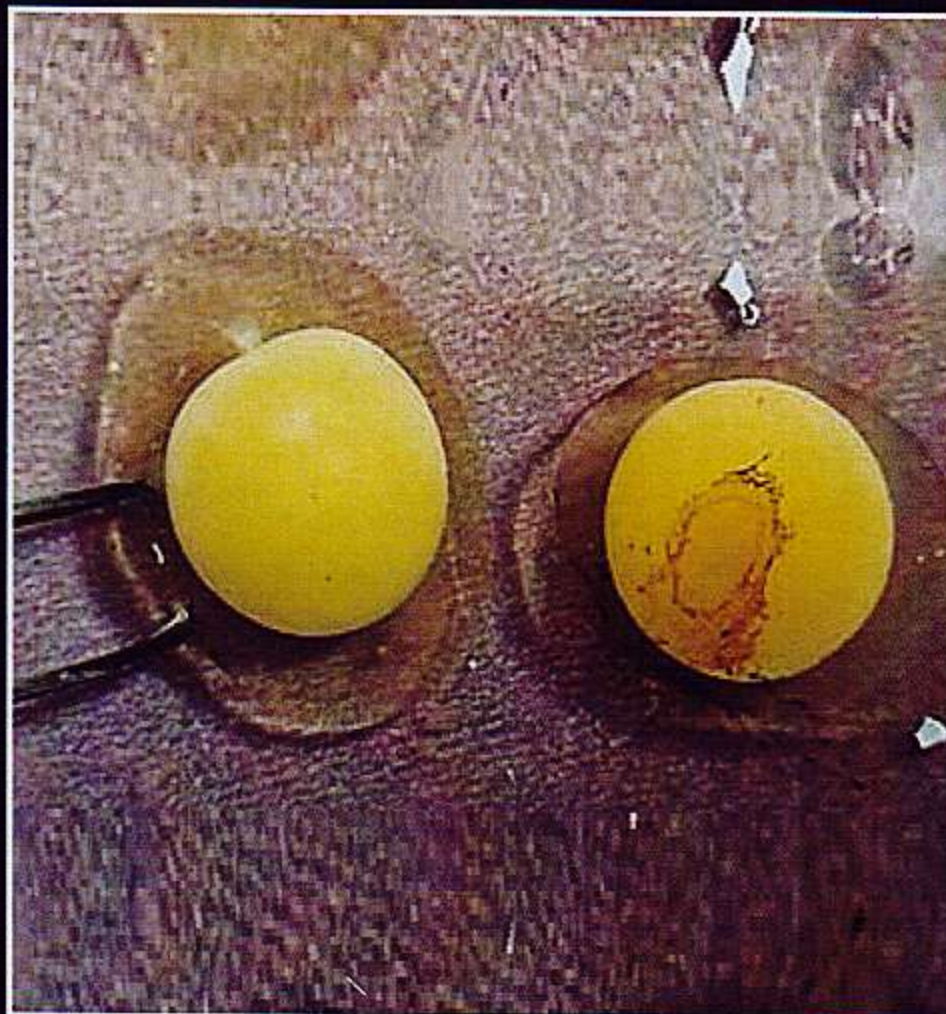


Development of Japanese Quail: From blastoderm to a complete chick

Compiled by

Mohd. Matin Ansari
Martina K
Gautham Kolluri
Jagbir Singh Tyagi
Rokade JJ
Adnan Naim
Ashok Kumar Tiwari



cari



ICAR-Central Avian Research Institute

Izatnagar-243122, Bareilly (U.P.)

Tel: 0581-2303223 ; 0581-2971806

Email: director.cari@icar.gov.in | Website: www.cari.icar.gov.in



Abstract: In avian, the development of an embryo in an egg outside the mother's body provides a distinct advantage, making chickens and quails ideal model organisms for studying developmental biology. Japanese quail is preferred over chicken due to its smaller size and shorter reproductive cycle. However, there has been limited research in India utilizing quail as a model. In this article, we present stage-wise developmental data for an in-house developed variety of Japanese quail, CARIUTTAM, and compared it with previously published studies.

Introduction: Developmental biology offers the opportunity to explore various organisms as models for studying the temporal and spatial aspects of growth and development. Avian embryos, in particular, play a crucial role due to the unique advantage of developing within an egg outside the host body. While chickens are commonly used as the primary model organism in avian developmental research, other important avian species such as ducks, quails, pheasants, guinea fowl, and turkeys have gained popularity due to their distinctive characteristics (Davey & Tickle 2007).

Quail embryonic development is a well-established model in developmental biology, offering valuable insights into vertebrate growth, differentiation, and organogenesis (Ainsworth *et al.*, 2010; Bai *et al.*, 2016). As a precocial species, the Japanese quail (*Coturnix japonica*) develops rapidly, with an incubation period of approximately 16–18 days, making it an ideal subject for research in embryology, genetics, and toxicology (Ono & Wakasugi 1983).

Similar to other poultry species like chicken, duck, turkey, goose and guinea fowl, the developmental stages of quail embryos follow a well-regulated process, starting with fertilization and cleavage, followed by gastrulation, neurulation, and organ formation (Sellier *et al.*, 2006). Similar to other avian species, quail development adheres to the Hamburger and Hamilton staging system, which categorizes embryonic growth into distinct morphological phases (Hamburger and Hamilton 1951). The accessibility of quail eggs for *in-ovo* observation and experimental manipulation has made them a preferred model for studies on chimeric grafts, transgenic, and developmental biology.

Studying quail embryonic development not only enhances our understanding of avian biology but also has significant applications in biomedical research, poultry science, and evolutionary studies. This study aims to document the developmental stages of Japanese quail variety developed at the host institute, named CARIUTTAM.

Materials:

- Fertile Japanese quail eggs (*Coturnix Coturnix Japonica*); CARIUTTAM
- Egg Incubator with automatic rotor
- Stereo-zoom microscope
- Fine forceps
- Scissors
- Petri-dishes
- Phosphate Buffer Solution
- Eosin Yellow stain
- Neutral Red stain

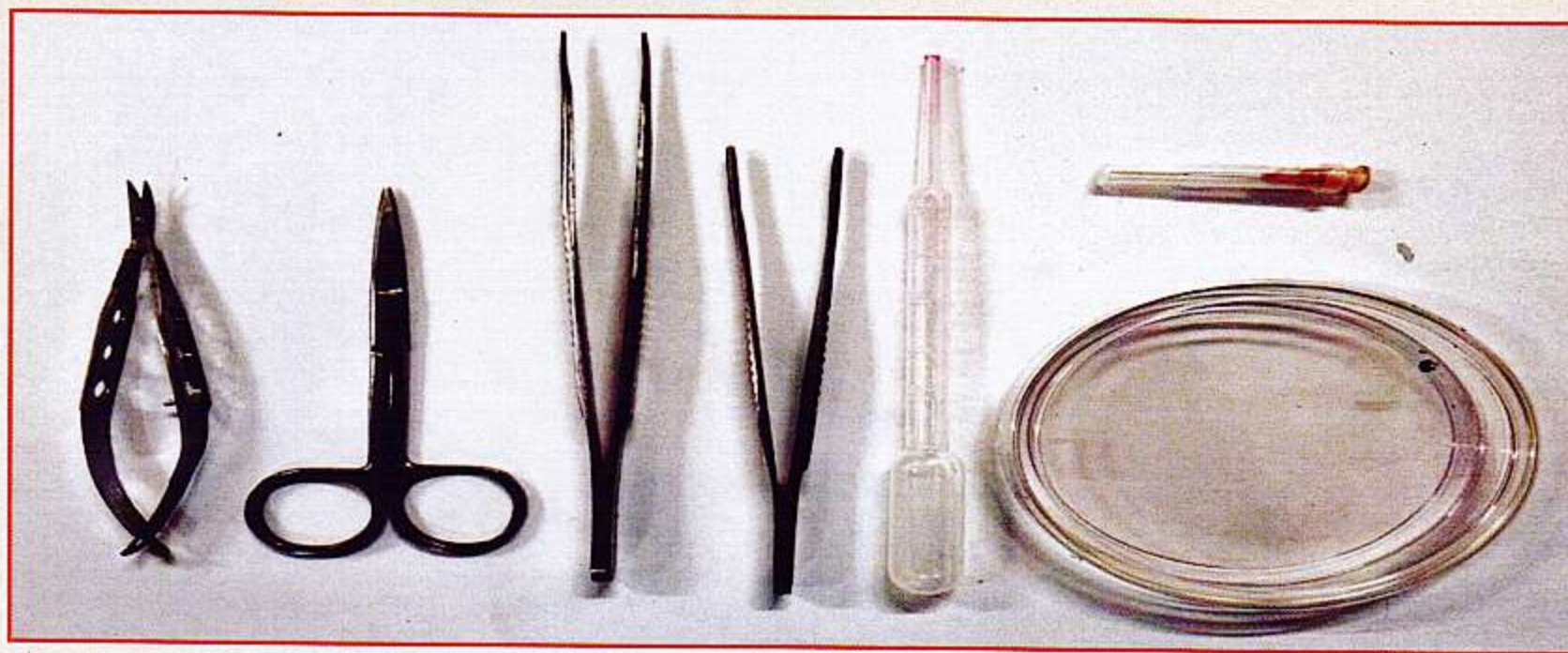


Figure 1: Instruments required for embryo collection. The image shows all the major instruments required for harvesting the developing embryo. A). Spring scissor B). Sharp end scissor C). Pointed forcep D). Blunt end forcep E). Pasteur pipette F). Petri Dish G). Needle

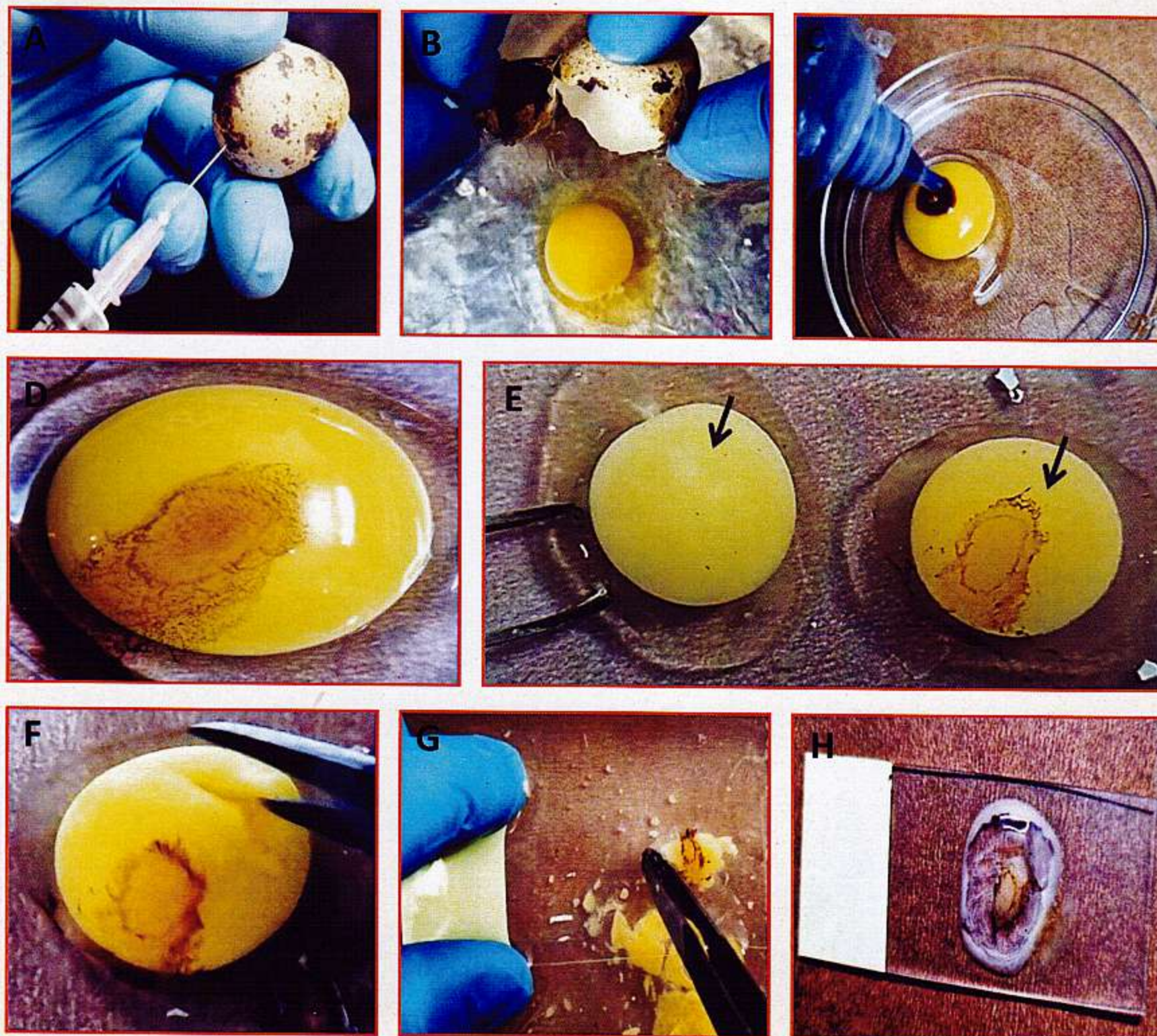


Figure 2. Collection of blastoderm and developing embryos from the quail eggs. A). Removal of the albumen from the eggs using syringe. B). Break open the egg and collection of yolk containing blastoderm or developing embryo in a petri-plate. C). Staining of developing embryo using Neutral Red dye. D). The neutral red stain the developing embryo making it easy to identify and harvest. E). The stained embryo is transferred to a tank filled with phosphate buffer solution. F). Holding the embryo with tooth-forcep, the embryo is cut in circle using a sharp scissor. G). The cut isolated embryo is transferred onto a glass slide dipped in the buffer. H). The harvested embryo is collected on a glass slide and observed under the microscope for imaging.

Methods:

Embryos were staged every 24 hours until hatching. The initial stages of the embryo development were images using the stereozoom microscope (Nikon). Embryos after Embryonic Development Day (EDD) 9 were photographed using the digital camera.

Preparation of embryo samples:

For studying the development of embryo, the embryos were collected every 24 hrs and the whole embryos were separated from the egg yolk, rinsed phosphate buffered saline and fixed in 10% formalin.

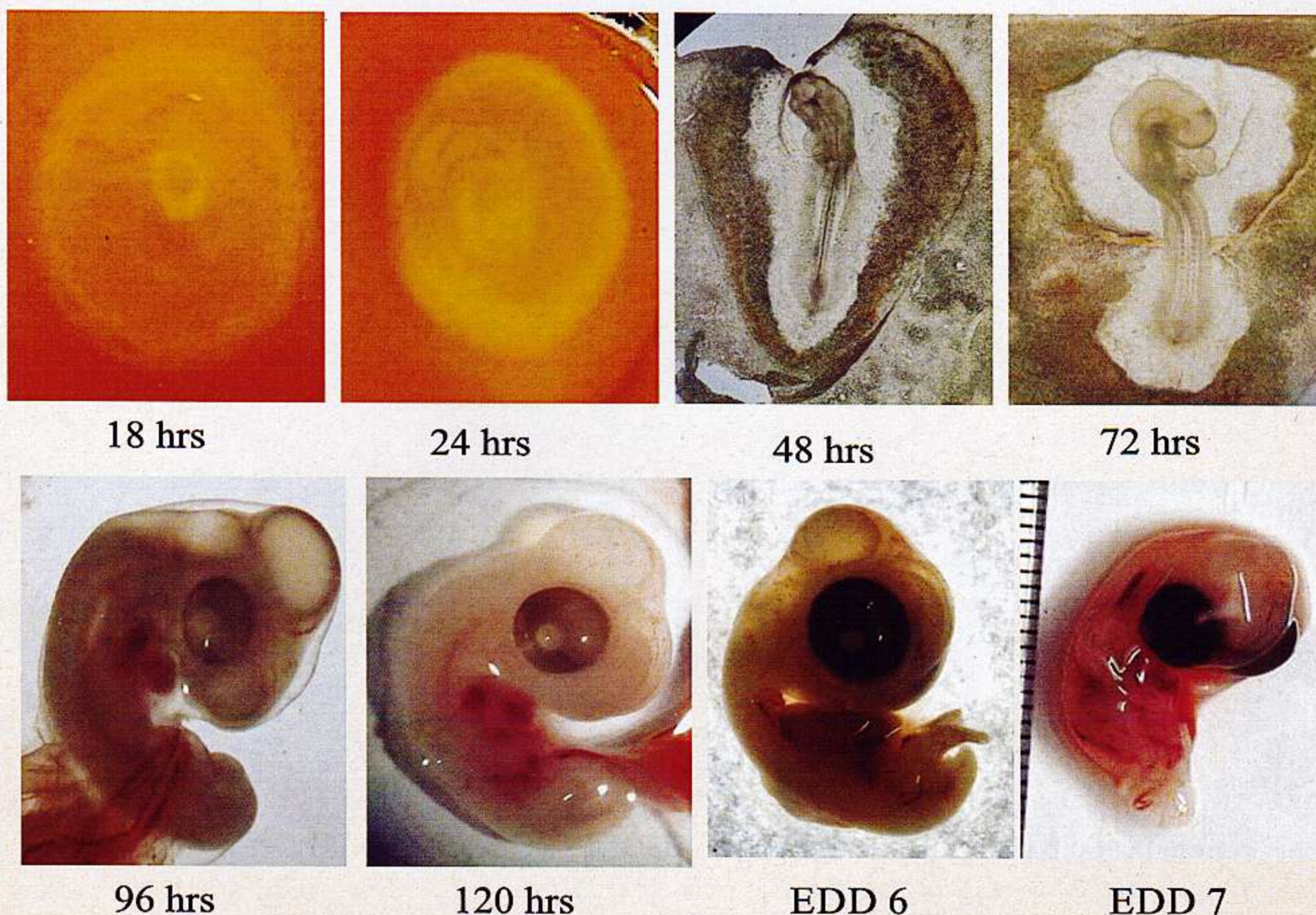
Photography and studying the embryo development:

The embryos were accessed by breaking the eggs and harvesting the embryos using the stereozoom microscope (Nikon). The embryos were collected on glass slide and stained using neutral red and eosin respectively. The images were captured using Zeiss upright microscope.

Figure 3: Filter paper ring method for harvesting embryo. A filter paper ring of suitable diameter was prepared and placed over the developing blastoderm. The yolk membrane was cut adjacent to the edges of the filter paper ring, which was lifted using the forceps and embryo was released on a glass slide for further study.



Developmental stages of J. quail



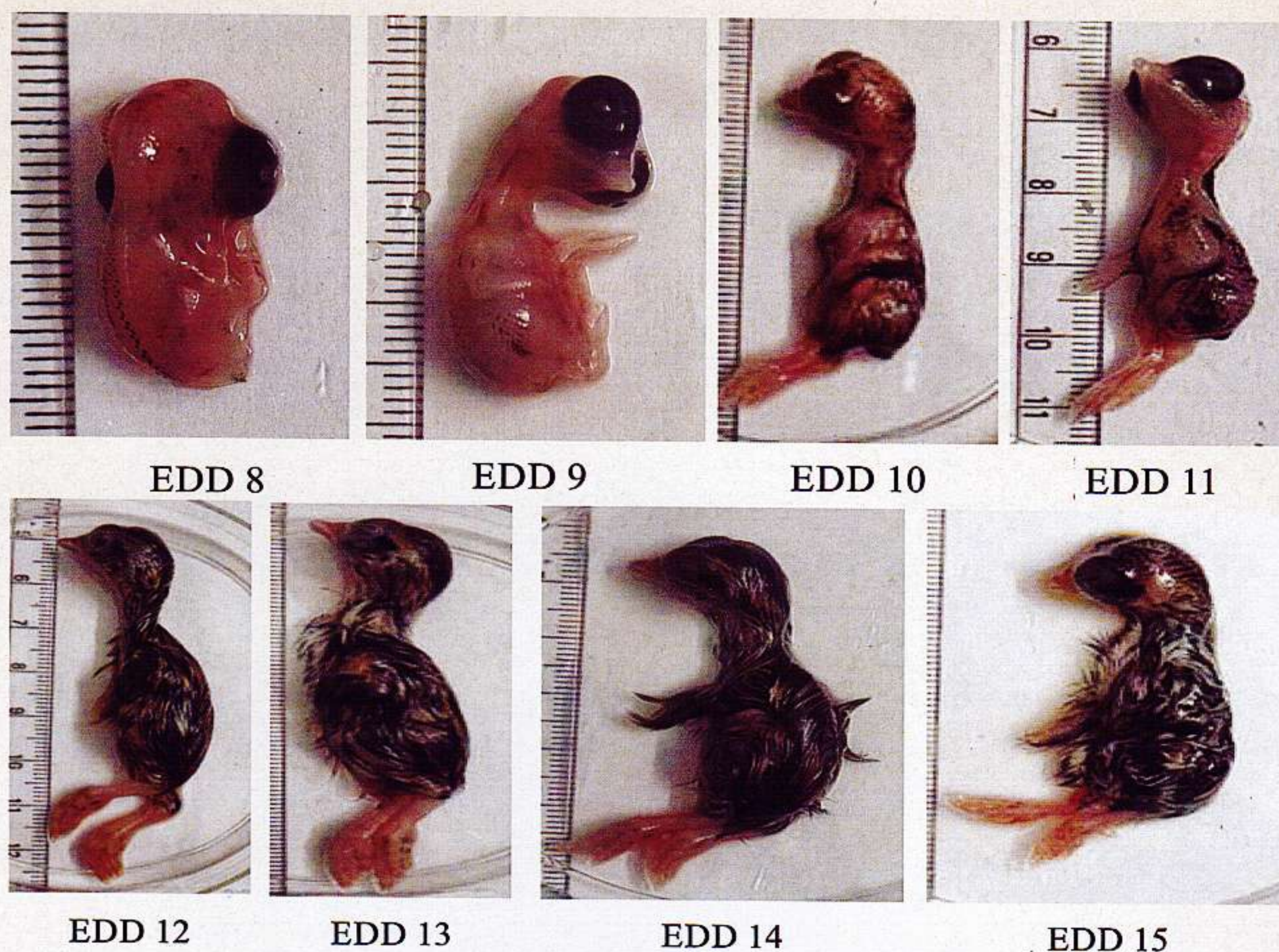


Figure 4. Developmental stages of *J. quail* (CARIUTTAM): At different time period of incubation, the morphological development of Quail embryo using CARIUTTAM as a model.

Table 1: Key developmental features summarized about CARIUTTAM (*J. Quail*) embryo development

S.No.	Embryonic Development Day (EDD)	Key developmental features
1.	EDD 1 (18 hr - 24 hr)	Presence of primitive streak (PS). Notochord and head fold was present
2.	EDD 2 (25 - 48 hr)	Presence of 1 - 16 somites.
3.	EDD 3 (49 - 72 hr)	Lack of pigmentation in eye, presence of distinct somites, first appearance of leg bud and wing bud
4.	EDD 4 (73 - 96 hr)	Presence of eye pigmentation, presence of limb buds
5.	EDD 5(97 - 120 hr)	Distinct knee and elbow joint in the limb buds
6.	EDD 6 (121 - 144 hr)	Presence of Egg tooth

7.	EDD 7 (145 - 168 hr)	Presence of scleral papillae
8.	EDD 8 (169 - 192 hr)	Brown pigmentation appeared. Eyelids starts to develop
9.	EDD 9 (193 - 216 hr)	First appearance of Black and Brown feather germs on the back bone
10.	EDD 10 (217 - 240 hr)	Presence of feather germs all over the body
11.	EDD 11 (241 - 264 hr)	Pigmentation was evident in feathers and at inter-tarsal joints.
12.	EDD 12 (265 - 288 hr)	Presence of pigmentation on toes. Increase in beak length of the embryo
13.	EDD 13 (289 - 312 hr)	The length of the feathers was increased
14.	EDD 14 (313 - 336 hr)	Embryo size and weight were increased
15.	EDD 15 (337 - 360 hr)	Maximum yolk utilization was observed, length and weight of the embryo was increased
16.	EDD 16 (361 - 384 hr) / Day of Hatch	Pipping / Hatching of the chick

Approximately 100 fertile eggs of Japanese quail were procured from the host institute and incubated at 38 degree Celsius and 70% relative humidity. As shown in figure 1, the embryos were collected from the incubated eggs by cracking the eggs and leaving them in the Phosphate Buffer Saline solution for 1-2 minutes then breaking the shell and releasing the embryo intact to the yolk sac in the Phosphate Buffer solution. We used Neutral Red to stain the embryo lying intact with the yolk sac for contrast and easy isolation of the embryo from the yolk. Sharp scissors were used to cut the embryo and releasing the embryo in the phosphate buffer solution. The embryo was cleaned for any residual yolk content and then was placed on the glass slide. The embryo on the glass slide was further stained with eosin yellow for distinct identification of somite and other tissue and organs. In figure 2, we harvested the early stage embryos using filter paper ring. The Whatmann filter paper rings were cut in circular shape using scissor and made a hole in the centre of the paper ring. The filter paper rings were autoclaved prior to use.

Results and Discussion:

The Hamburger and Hamilton (1951) and Eyal Giladi and Kochav (1976) staging of embryos using chicken as a model organism, were used for reference of comparing the developmental stage of quail embryos. Developmental stages in Blue breasted quail was also used a reference to compare progress in development of CARIUTTAM embryos (Nakamura *et al.*, 2019). Figure 3, shows the different stages of development of the J. quail CARIUTTAM embryo.

We imaged the embryo from EDD 1 – 16. We observed hatching of 75% of the chicks on EDD 16, whereas different authors have considered EDD 17 or EDD 18 as the day of hatch.

Conclusion:

The present study summarized and records the different morphological developmental stages of CARIUTTAM, a variety of Japanese quail developed at the host institution. The data generated will be used as a reference for the students research personnel & other concerned involved in the developmental biology of J. quail.

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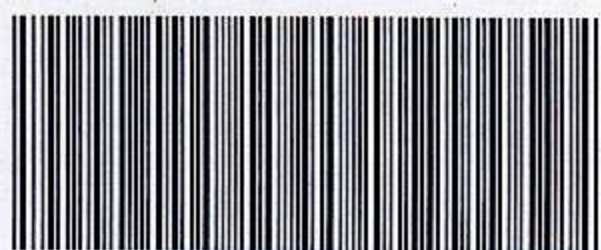
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**ICAR-Central Avian Research Institute,
 Izatnagar**

Tel: 0581-2303223 ; 0581-2971806
Email: director.cari@icar.gov.in
Website: www.cari.icar.gov.in

