Original Article

Reproducing Windowing Technique for Naked Eye Observation of Chick Embryo Morphogenesis

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Background: Human embryos are not easily available for teaching purposes. Embryology teachings rely mostly on simulators. Fantasying the embryological stages falls far behind in giving an exact picture of the events to undergraduate medical students. This study uses chicken embryo biological models for observing naked eye development stages. These model systems not only prove to be a realistic approach to the subject of embryology but also provide an ideal experimental medium for research.

Objective: To device a model system for easy access to the developing embryo, maintaining a natural developing environment as close as possible.

Study Design: Descriptive case series.

Materials & Methods: This study was conducted in the Anatomy department of of Islamabad Medical and dental college, Islamabad Pakistan. Small windows were made on the shells of fertilized chicken eggs. One edge of the circular window was left hinged as a lid to the shell. The eggs were incubated under standard conditions. The developing embryos were daily taken out and day wise development was studied by just lifting the lid without sacrificing the embryo.

Results: In this study three batches, each comprising of 6 fertilized eggs were traced for developmental mile stones via windowing technique. Formation and growth of the embryonic membranes, central nervous system, circulatory system, eyes, limbs, skin, wings and folding of the body were directly observed and the developmental time line also traced in 100% embryos.

Conclusion: This system provides a media for in ovo experimentation for the researchers without much manipulation to the natural environment.

Key words: Embryogenesis, albumen, central nervous system.

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Introduction

Embryology is a branch of biology that deals with formation, early growth and development of the living organism.[1] It is a subdivision of Anatomy[2] and is taught as a compulsive subject to under and post graduate medical students. Moreover, it has always remained an essential area of research among scientists.

Learning anatomy utilizes various teaching tools, like cadaveric dissection, models, and microscopic slides in addition to traditional lecture hall teaching. Understanding the embryological development stages has always remained a problem for the medical students.[3] Unlike human cadavers, embryos are not easily available for teaching purposes. Whereas visual learning and practical approach has emerged as a modernized teaching methodology today[4], embryology has to rely on a few museum specimens, models and animated video CDs. Keeping in view these educational hindrances for embryology, this study uses chicken embryo biological models for observing naked eye day to day development stages of embryos.

The pace of embryological research has amplified exponentially in the 20th century even leading to prolonged ethical disagreements[5].Chick embryos

have proved an effective animal model substitute for these researches. [6,7] Embryogenesis in chicks involves developmental processes equivalent to those occurring in mammals, but takes place at higher rate.[8] True growth takes place during morphogenesis in which the embryo and its developing organs increase dramatically in size, similar to the situation in the human embryo.

Keeping in view the potential application of chick embryo model system in medical research and non availability biological specimens for study purposes, this study was designed to device a model system for easy access to the developing embryo, maintaining a natural developing environment as close as possible.

Materials and Methods

This descriptive case series was conducted in the Anatomy department of Islamabad Medical and dental college, Islamabad Pakistan. Three batches, each comprising of 6 freshly hatched fertilized chicken eggs were collected via simple random sampling from the Poultry Research Centre, Rawalpindi. The eggs were incubated under standard conditions of 37.5°C and 60-70% humidity 9. After first 24 hours of incubation the eggs were processed in a careful manner (Figure I, A-H) to make a window over the shell of the eggs. At one time, a batch of six eggs was taken out of incubator maintaining their horizontal position as they were carried to the bench. They were placed in the horizontal position in a pre cleaned egg holder and were wiped with 70% ethanol soaked gauze before making the windows (Figure I-A). A piece of wide Scotch tape was pasted along the long axis of the eggs, so that the tape covered most of the "top" of the eggs (Figure I-B). The rounded ends of the eggs were covered with a small piece of tape (approx 1cm2). The tape covered rounded ends were punctured. Pointing the needle down at about an angle of 45o, 3-5 ml of albumen was withdrawn (Figure I-C). This allowed the embryos to move away from the upper surface of the egg, where the window was to be cut. This small hole was sealed off after albumen extraction by another tape piece. With the tip of a scissor, the taped-covered tops of the eggs were punctured about a half an inch off center (Figure I-D). An INCOMPLETE oval opening was cut by pulling up the scissors keeping as far away from the embryo and vitelline envelope as possible (Figure I-E&F). The oval cut was not completed end to end, leaving a hinge type shell lid on the top of the eggs (Figure I-G). This shell lid was closed off over the window by means of a tape piece (Figure I-H). The eggs were replaced in the incubator. They were removed from the incubator daily for not more than 5 minutes/per session and were observed, photographed and videoed by merely opening up the lids and securing after observation (Figure II-A-F). All embryos survived the days of early embryogenesis, and proceeded with further growth & development, latest reaching the 19th post incubation day (Figure II-F).

Results

The developmental process of the embryos observed directly by unaided eye in these windowed eggs was correlated with Hamilton-Hamburger (HH) stage[10] of development. The developmental milestones of the embryos were traced from early to advanced well developed stages in all the embryos. At 33 hours the embryos resembled the HH-10 stage while at days 5, 9 12 and 19, HH stages 25, 35, 38 and 45 were reached respectively.

In this experiment, formation and growth of the embryonic membranes, central nervous system, circulatory system, eyes, limbs, skin, wings and folding of the body were directly observed and the developmental time line also traced and correlated to HH staging pattern.

The progressive stages of development as noted in the embryos were comparable with the Hamilton-Hamburger's stages. Theses stages and actual observations are summarized in **Table I** and **Figure II** illustrates some of the observed features.

Table I: Periodic embryonal morphogenesis correlated with Hamilton Hamburger (HH) Stages

Time of Observation (Hrs/days)	Correlating HH stage	Observed developmental mile stones
33-38 hours	(HH 10)	Vasularized blastoderm, 8-10 somite stage, 3 primary brain vesicles.
Day 4.5 – 5	(HH-25)	Cardiac bulge, 5 brain vesicles, optic cup, dorsal contour from hindbrain to tail is a curved line.
Day 9	(HH-35)	Limbs lengthened Phalanges formed. Eye development almost complete.
Day 12	(HH 38)	Appearance of hair line, Eye lids, head and limbs completely formed, beak formed, thick vasculature.
DAY 19	(HH 45)	Dead embryo taken out of shell and observed for developmental mile stones. (Wings completely developed; body shape and form complete, and yolk sac regressed back into the abdominal cavity.

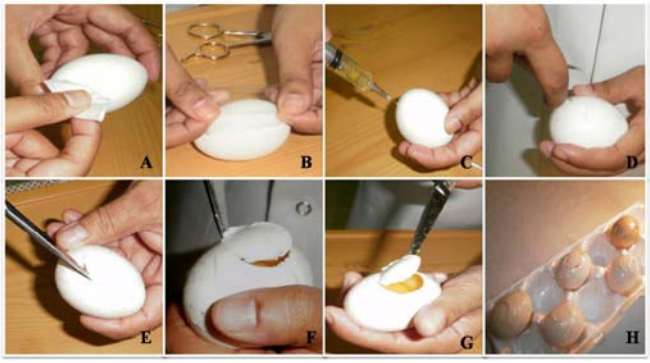

Figure I: Steps of Windowed egg technique (Description given in the section of materials & methods)

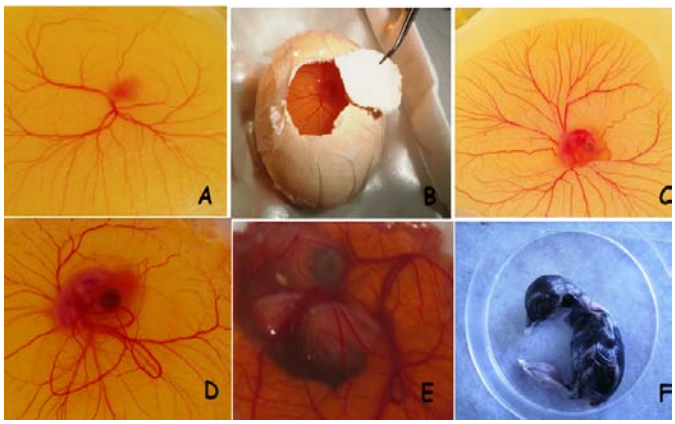

Figure II: Naked eye observation of stage wise chick embryo morphogenesis:

A: Vasularized blastoderm.
B: Cardiac bulge, 5 brain vesicles, optic cup, dorsal contour from hindbrain to tail is a curved line
C: Close up of photograph "B" (Early organogenesis).
D: Limbs lengthened, Phalanges formed. Eye development almost complete.
E: Appearance of hair line, Eye lids, head and limbs completely formed, beak formed, thick vasculature.
F: Dead embryo taken out of shell and observed for developmental milestones (Wings completely developed, body shape and form complete, yolk sac regressed back into the abdominal cavity).

Discussion

Studying the real-time developmental process has always remained a challenge, which has now been met with the construction and establishment of living model systems allowing moment to moment observation of the developing embryos. Efforts are being made for improving technology for studying the embryology in a more comprehensive fashion.[11] Recently computer-based video imaging system has been established for analyzing critical phase of cardiac looping in live chick embryos.[12]

Many in vitro technologies[13] for chick embryogenesis have been introduced earlier for interventional studies followed by more advanced ones. Researches involving injection of drugs[14] and other agents [15] into the fertilized chicken eggs, where on one hand have a more difficult approach to the developing embryos; on the other suffer a trauma stress bias factor.

Although windowing substantially reduces the hatchability of eggs containing early embryos[16], this technique has the advantage of providing easy access to the embryo and extra-embryonic membranes for the observation of morphogenesis and growth as well as for the application of different agents under study. The ease of accessibility of the embryo has allowed for experiments to map cell fates using several approaches, including chick quail chimeras and focal dye labeling, molecular perturbations of several types, and introduction of plasmid DNA using in ovo electroporation.[17] These experiments have yielded important data on the development of the central and peripheral nervous systems.

The windowed egg technique can be compared to shell less culturing as described in our previous study[18], with a few added advantages. This method preserves the simulation to natural developmental process providing better experimentation models. There are less chances of contamination and it costs less with no expenditure on cultural accessories.

This embryological stages observed in this model system can be good specimens for teaching embryology in anatomy museums.

This naked eye observation system is accurate, cost effective and easily reproducible for research and academic purposes.

Conclusion

Windowed eggs technique could be utilized in the embryological anatomy classes, as this technique would be very helpful for the undergraduate students in comprehending the stages of embryo development. This cost effective, easily reproducible technique, which follows closely the normal in vivo developmental milestones with minimum manipulation with the natural environment, can also be adopted by the researchers in experimental studies.

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