

# Pattern of Blood Vessels Development at First Seven Days of Incubation of the Wild Helmeted Guinea Fowl (*Numida meleagris galeata*). Gross Approach

N Wanmi<sup>1\*</sup>, OM Samuel<sup>2</sup>, N Plang<sup>3</sup> and BE OKE<sup>4</sup>

<sup>1,2</sup> Department of Veterinary Anatomy, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State.

<sup>3</sup> Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Jos, Plateau State.

<sup>4</sup> Department of Theriogenology, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State.

## Abstract

The wild helmeted guinea fowl has in recent time been used for research in the field of anatomy because of its peculiarity from other domesticated species of avian. Eggs of the wild helmeted guinea fowl are considered to be nutritious and has been used for medicinal purposes in some rural settlements in Nigeria. Eggs of the wild helmeted guinea fowl were purchased from hunters and taken to the National Veterinary Research Institution (NVRI) for incubation. Immediately fresh eggs were purchased, it was kindle using high powered light because of its thick egg shell and only eggs which have not started developing will be incubated and that marks the first day of incubation. On day 3 of incubation, large patches of appears redder on the surface of the egg yolk. These congested sites, develop around portion were future embryo will formed. Blood vessel were first, observed on day 4 of incubation and as days on, as embryo increases in size, blood vessels increase as well. The point of embryo implantation is evident first; by formation of congested areas and most importantly, a single zone of circular red rim. This mark the point of implantation. Blood vessels of the wild helmeted guinea fowl develops from the surface of the egg yolk, which appears initially as small strips of line. Blood vessels connects to the site of embryo implantation on day 3 of incubation. Blood vessel is the first structure to be form prior to the manifestation of the embryo.

**Keywords-** Days, Ontogeny, Seven, Vessel,

**\*Corresponding Address:** Wanmi Nathaniel, Department of Veterinary Anatomy, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria

## I. INTRODUCTION

The helmeted guinea fowl (HGF) (*Numida meleagris galeata*) is a native to Africa and belongs to the Phylum, Chordata; Subphylum, Vertebrata; Class, Aves; Order, Galliformes; and Family, Numidae. It is widely distributed in the Guinea Savannah vegetation zone of Nigeria [1] and estimated at 44 million in captivity [2]. In Nigeria, two types of guinea fowl species are found; *Numida ptilorhycha* that is indigenous to the Southern part while *Numida meleagris* is domiciled in the Northern part but is spreading to other small-holder farming areas [3]. Some people keep guinea fowl out of curiosity and as “watch animals” around homestead because they have excellent eye-sight, a harsh cry, and they shriek at the slightest provocation [4]. They are also kept for income generation [5], and for the control of snakes, mice and ticks [6] thus, encouraging its production. The increase in guinea fowl production has led to the development of informal traders who buy and sale the birds for breeding and consumption, especially during festive seasons [7].

Generally, the first step in the formation of blood vessels is the emergence of their inner lining cells, the endothelial cells (EC). These cells then associate into tubular structures and other cells types, notably smooth muscle cells or pericytes, organize themselves around them to make the vessel wall [8]. Structural specializations such as tight junctions in vessels of the blood-brain barrier or regionalized. Adhesion molecules

are acquired by differentiating EC. The development of new blood vessels is an uncommon physiological event in adults. During ontogeny endothelial cells must clearly emerge *de novo*. In this process, designated as vasculogenesis [9] means of detecting these cells as soon as possible after their determination are crucial. Early investigators explanted the whole chick embryo *in vitro* and observed the development of the vascular tree by following the movements of red cells [10]. Similarly, real progress came from monoclonal antibodies that recognize isolated endothelial cells with great sensitivity [11]. These antibodies were obtained against immunogens from the quail species. They actually recognize the hemangioblastic lineage, i.e. endothelial and hemopoietic cells (HC). These antibodies gave a boost to research on the embryonic emergence of endothelial cells, making the avian embryo a privileged model for this study. Despite elaborate information are available on the development of blood vessels in other species of chicken, there are dearth of information on the pattern of blood vessels formation during the first fourteen days of development in the HGF. Work done on the development of the guinea fowl includes; [12] structural organization of the optic lobe of grey breasted helmeted guinea fowl (*Numida Meleagris Galeata*) at Pre-Hatch Study, [13] skeletal development of guinea fowl, [14] on the development of GIT in guinea fowl and [15] cortical development of the chicken cerebellum. However, basic information on the pattern of blood vessel formation in the wild grey breasted helmeted guinea fowl is not currently available, hence the need for this investigation.

## II. MATERIALS AND METHODS

### A. Experimental eggs

Fifty six (56) fertilized guinea fowl eggs were purchased from National Veterinary Research Institution (NVRI) Vom, Jos, Plateau State, Nigeria. The eggs were transported to a hatchery in Jos and incubated using their standard incubation guide. During incubation, the eggs were turned regularly (minimum of three times) each day for the first 14 days. Four (4) eggs (two eggs per day) for pre-hatch study were collected daily from day one of incubation up to day fourteen which of incubation. An opening was made on the large air space area and the entire egg dropped into a labeled container of 10 % buffered formalin for proper fixing.

### B. Extraction of embryo from egg shell

This was done at pre-hatch using a scalpel blade and clean transparent dish. The blunt side of the scalpel blade was used with the egg held on the palm, and a gentle tap was made on the egg until a crack was formed. Then, the crack was gently widened manually and the embryo collected in a transparent dish.

### C. Gross

Photographs of the egg yolk where developmental processes begins were taken using digital handheld Microscope (Magnification 1000x, 5x Zoom, 3D stand high speed DSP).

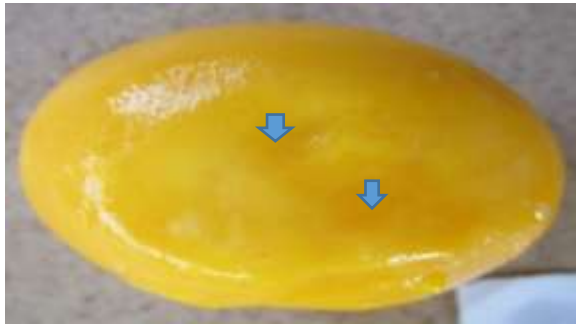
## III. RESULTS

Immediately after incubation within 24 – 48 hours, egg yolk appears consistently yellow throughout its sphere. At this stage of development, it was void of blood vessels nor site of congestions, (Plate 1).



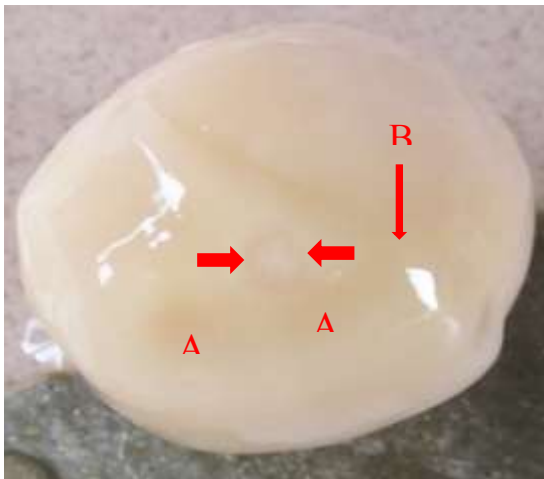
**Plate I: Uniform consistency yellow-coloured egg yolk (Day 1 pre-hatch). X12.1**

At day 3 of incubation, there were large focal points that appeared like areas of hyperemia, with blood vessels pooling towards the various areas. Smaller strip-like lines first appears draining from areas of congestions. These strip-like areas are future site of blood vessels formation on the surface of the egg yolk (Plate II).



**Plate II: Egg yolk indicating, Arrows; Areas of blood clot on the yolk surface (Day 3 pre-hatch). X12.1**

At day 4 of pre-hatch development, sites where future embryo will be formed was shown by a single rim that was reddened like a circled zone with the center within the rim taking the consistency of the egg yolk. At this stage, the strip-like regions that appeared in day 3, turn out to form smaller blood vessels. These minute blood vessels tend to converge towards the encircle zone. Zones that formed hyperemic areas at this stage, begins to resolve into numerous strips of reddened lines. Grossly, at day 3, blood vessels formed might have not develop link with site where embryo will be form (Plate III).



**Plate III: Yolk of helmeted guinea fowl indicating: A; Points of blood clots formation, B; Blood vessel, Between Arrows; An encircled Point, future site for embryo development, (Day 4 pre-hatch). X12.1**

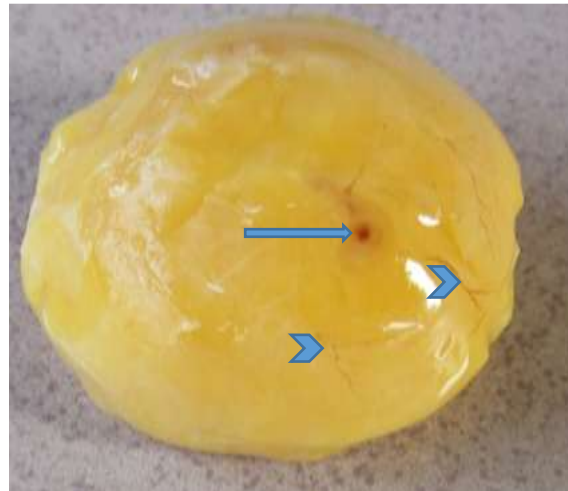
Three basic gross features were observed on day 4 of incubation; first time blood vessels establish

with embryo at various regions. Circled portion turn to site for embryo formation evidence with a placode within the cephalic region and numerous blood vessels having a tributary-like ramifications around the zone of embryo formation. The surface circumference of the egg yolk were highly reddened, mostly towards the point of embryo formation, resulting from branching of

smaller blood vessels, converging towards the site of embryo formation. Blood vessel of the HGF

is formed from surface structure of the egg yolk and subsequently, will become the embryo future

blood vessels. (Plate IV).



**Plate IV: Embryo of the helmeted guinea fowl showing: Arrow; Le as placode and J-shaped Embryo forming, Arrowheads; blood vessels, (Day 5 of incubation). X12.1**

Day 6 pre-hatch development depict prominent elaboration of large blood vessels on the egg yolk surface. These vessels links to the embryo by large stump that appears like arbor vitae, encompassing the embryo. This shows the egg yolk is the primordial site where nutrients needed

for the viability of the embryo is been derived (Plate VI).



**Plate V: Developing embryo of the helmeted guinea fowl showing (A): Arrow; an oval shaped embryo, B; Blood vessels, (Day 6 pre-hatch). X12.1**

#### IV. DISCUSSION

Ornithological studies, most especially, on avian embryos date back to Aristotle, and even further back, to the Egyptians. The avian model has played an important role in establishing the foundations in blood circulation research and since then, many species of avian is been used in comparative embryology [16]. During ontogeny, endothelial cells must clearly emerge. To study this process, designated as vasculogenesis, means of detecting blood vessels as soon as possible after their determination are crucial [17].

The egg yolk of the wild HGF was seen to be consistently yellow, but other forms of coloration has been reported in the domestic HGF to be milk-like to pink colour [12]. The egg yolk plays a significant role during pre-hatch development of the embryo by primarily providing it with the necessary nutrients required for the development of the embryo [18].

At day 3 of development, blood clot was first noticed, and by the fifth day, blood vessels were prominent. In the helmeted guinea fowl, one of the first organ systems to develop is the vascular system with blood vessels being visible on day 4 of incubation. Blood islands were seen soon after day 3 pre-hatch and the circulation was established on day 2 of incubation in the chicken [19]. This supports the fact that vascular system is the

first to be formed during embryogenesis of the galliformes. The embryo of the helmeted guinea fowl appears first on the fourth day of incubation with a rostral projection of the brain and an eye spot referred to as lens placode, and a caudal tail with a stripped middle part as the body. This developmental order was reported in the chicken by [20]. [14] reported that the GIT first developed on the 8<sup>th</sup> day of incubation and an osteogenic development in grey breasted guinea fowl on day 10 [13]. This indicates that during avian embryogenesis, the circulatory system is the first organ to appear and subsequently, the nervous system develops alongside with it in the Galliformes.

#### V. CONCLUSION

Blood vessel develop from egg yolk and will be the future vessel that will be incorporated into the body of the HGF for circulation. As embryo increases in size, blood vessel enlarges to meet the body demand for nutrients. Blood vessel are more on the side where embryo is formed than other portion void of embryo formation.

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#### CONFLICTING INTEREST

No conflict of interest

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