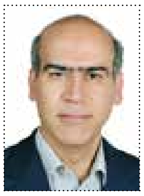
Morphologic Evaluation of the Fertilized and Unfertilized Ostrich Egg during the Early Stages of Development (MRI Analysis)

Bahador Shojaei [1*], Hadi Tavakoli [2], Mohammad Javad Aghasizade [1], Hamed Reza Sayyah Badkhor [3]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
3. Technician of Kowsar MRI Center, Imam Reza Hospital, Mashhad, Iran.



Bahador Shojaei is associate professor of Veterinary Anatomy. He teaches comparative anatomy and embryology at Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran. His research interests are imaging anatomy and morphogenesis.

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ABSTRACT

Introduction: The magnetic resonance imaging (MRI) technique permits to monitor the development of the embryo in vivo without causing any developmental defects. Although increasing application of MRI imaging are predicted in veterinary sciences, there is little information available about MRI studies of avian embryos and their eggs. The objective of the present study was to evaluate the ostrich embryonic development in the first 18 days of incubation using the MRI technique.

Methods: Fertilized and unfertilized ostrich eggs were incubated in standard condition. The eggs were removed from the incubator on days 2 to 18 post-incubation and exposed to 1.5 Tesla magnetic fields.

Results: The results showed that the structures of unfertilized eggs remained fairly constant over the 18 days of incubation but it was a significant change in fertilized eggs from 4th day. At early stages, the considerable structures of the eggs such as wight/yellow yolk, albumen, air cell, latebra and neck of the lateral were revealed very clearly but the embryo and blood vessels could not be visualized until the day 6 post-incubation. Some details, such as eyes and amniotic sac, were also seen on day 10 of development. At later stages, the spherical shape of the yolk becomes completely distorted, it was distributed across the middle of the egg and the yellow and white yolk layers were disappeared.

Conclusion: Based on the findings, it is concluded that MRI is a suitable technique for following the development of a live ostrich embryo and its egg components at the first half of the incubation period.

*** Corresponding Author:**
Bahador Shojaei, PhD
Address: Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
Tel: +98 (913) 3418537 Fax: +98 (34) 33257447
E-mail: bshojaei@uk.ac.ir, b_shojaei@yahoo.com

1. Introduction

During recent years, efforts to improve anatomic studies without causing hazard to the embryo have been relatively successful. For this purpose different imaging methods have been used in different studies which intactness of live subject has been important [9, 13, 15, 19, 22, 27]. MRI is a sophisticated computerized imaging technique, which has been a clinical diagnostic tool since 1976 [12]. Until recently, MRI has had limited application in veterinary medicine due to the expense of the imaging unit, special equipment for animal handling and need for skilled staff, but during the past decade it has an increased role as a diagnostic option in different animal species including birds [7, 18], equine [2, 23] and dog [4, 5]. According to the process of image acquisition, Sonography and MRI are established methods for non-invasive embryo studying [16, 27], but egg shell does not permit operator to gain valuable data by the sonography of the live avian embryo [8].

Several studies have focused on MRI to explore the interior components of the fertilized egg in the chick and quail [1, 6, 7, 9, 10, 11, 15, 16, 20, 24, 25, 26]. Ostrich incubation period is nearly double of mentioned birds, so it is a suitable avian model for comparing the developmental events of the egg components from chronological point of view. Moreover the percentage of the main components of the ostrich egg (yolk, albumen and shell) differ from the chicken [21], so different type of their consumption expected throughout the embryonic period. The current study aimed to investigate the developmental changes in the extra and non-embryonic regions of the fertilized ostrich embryo using the MRI technique in the first half of the embryonic period. In this regard, the normal profile of the non embryonated ostrich egg were also assessed during this period.

2. Materials & Methods

Egg

A total of 10 ostrich eggs (Canadian breed) (seven fertilized and three unfertilized eggs) with the average weight of 1510±25 g were obtained from a local breeder farm. In this farm, birds were kept and grown up under the standard condition of breeding.

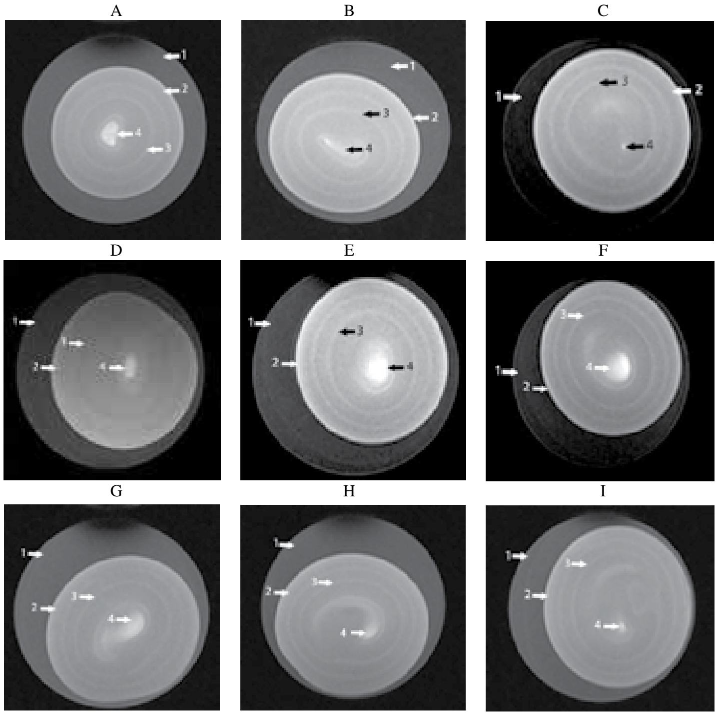

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Figure 1. Transverse MR images from the whole unfertilized ostrich egg (T1W). (A) Day 0; (B) Day 2; (C) Day 4; (D) Day 6; (E) Day 8; (F) Day 10; (G) Day 12; (H) Day 16; (I) Day 18: 1-Albumen, 2-Previtellin membrane and underlying yellow yolk, 3-White yolk, 4-Latebra

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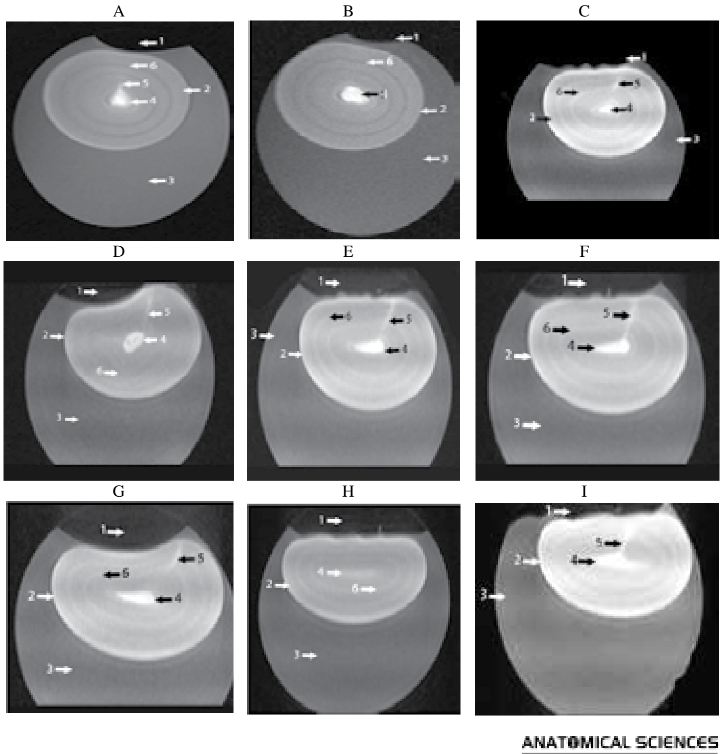

Figure 2. Sagittal MR images from the whole unfertilized ostrich egg. (A) Day 0; (B) Day 2; (C) Day 4; (D) Day 6; (E) Day 8; (F) Day 10; (G) Day 12; (H) Day 16; (I) Day 18: 1-Air cell, 2-Previtellin membrane and underlying yellow yolk , 3-Albumen, 4- latebra, 5-Neck of Latebra, 6- White yolk

Experimental protocol

Eggs were incubated at 36.4ºC and 18.5% relative humidity. The eggs were fumigated and pre-warmed in the incubator for 12:00 h at 25°C, just before the incubation period. The day the eggs arrived to the incubator was designated as day 0 and they were imaged using 3D T1W and T2W sequence on days 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 post-incubation (PI). The eggs were removed from the incubator and imaged transversely and sagittally in an egg holder inside the radio frequency resonator of the MRI Unit (1.5 tesla, symphony, Siemens). After imaging, the eggs were immediately returned to the incubator in the same orientation. Data were analyzed using syngo-pack imaging PC-based software.

3. Results

Ten eggs were studied over an 18-day period. Consecutive images were acquired pre-incubation (day 0) and on days 2, 4, 6, 8, 10, 12, 14, 16 and 18 post-incubation (PI). Representative images are shown in Figures 1 to 4.

Figures 1 and 2 display respective transverse and sagittal images of the whole unfertilized ostrich eggs. These images show how the structures of the unfertilized eggs did not have any significant change during 18 days of experiment.

Figures 3 and 4 show transverse and sagittal images of the fertilized eggs, through the first 18 days of ostrich embryo development. The yolk, albumen, air cell, latebra and the neck of the latebra were similar in fertilized and unfertilized eggs at day 0 (Figures. 1A, 2A, 3A and 4A). The spherical yolk with its yellow and white layers was seen in the center of the egg. It was surrounded by the albumen. The air cell was located at the large end of the egg and shaped the upper surface of the yolk and albumen as a concave surface. The latebra was seen in the center of the yolk as a high resonance mass and its neck extended upwards towards the blastoderm/blastodisc.

No significant change in the structure of the fertilized eggs was perceptible at day 2 PI.

At day 4 PI, the yellow and white yolk layers of fertilized eggs began to disappear. Latebra and the neck of the latebra were seen without any structural changes.

By day 6, significant changes in the shape of the yolk were seen in the fertilized eggs. Spherical shape of the yolk was going to become ellipsoid, the yellow and white

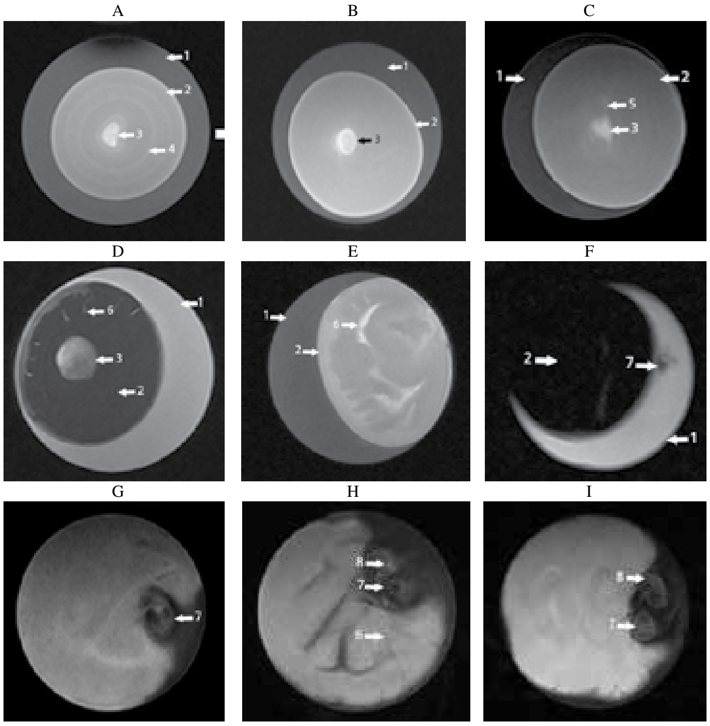

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Figure 3. Transverse MR images from the whole fertilized ostrich egg during the embryonic development. (A) Day 0; (B) Day 2; (C) Day 4; (D) Day 6; (E) Day 8; (F) Day 10; (G) Day 12; (H) Day 16; (I) Day 18: 1-Albumen, 2-Yellow yolk, 3-Latebra, 4-White yolk, 5-Neck of latebra, 6-Embryonic vessels, 7-Head of the embryo, 8-Body of the embryo

yolk layers were completely disappeared and blood vessels were observed on the surface of the yolk (Figures. 3D and 4D).

At day 8 PI, in the fertilized eggs, the neck of the latebra became distorted and less distinct. The embryo and blood vessels were seen on the surface of the yolk.

By day 10 PI, the yolk has moved up so that it was nearly no albumen touching the air cell. The eyes of embryo were distinguished clearly. Amniotic sac was seen vaguely around the embryo. Vascular belt was growing up in the distal third of the yolk.

Figure 4G shows distribution of the vascular belt across the middle of the egg at day 12 PI. This arrangement was seen in the subsequent days PI (Days 16 to18 in plates 3H–I) and prevented observer from seeing the border between the yolk and albumen. At day 12 PI the body of the latebra was no more observable. From day 12 on the visible changes were growing of the embryo and amnion, appearance of limbs and segments of the body.

4. Discussion

Most MRI studies on avian embryos have investigated the development of the embryo [20, 24, 25], while the extra-embryonic and the nonembryonic components within the egg have attracted less attention [9, 11]. Changes of the main non embryonic egg components during incubation can give us useful information for interpretation of developmental events [3]. Although the embryo, its eyes and body segmentation were recognizable as soon as the day 8, 10, and 12 PI respectively, in this study we focused mainly on the developmental changes of the extra embryonic components of the ostrich egg.

The yolk of the laid egg is enclosed in the vitelline membrane. Its center is marked by a ball of white yolk, the latebra, and from this a column of white yolk, the neck of the latebra, extends upwards to join with a disc of white yolk, the nucleus of Pander, which lies beneath the blastoderm. The yolk arranged in a series of concentric rings around the latebra which were of yellow and white yolk alternately [3]. From the latebra a column of white yolk, the neck of the latebra extends upwards to join with a disc of white yolk, the nucleus of Pander, which lies beneath the blastoderm [3]. The yolk shape and its posi-

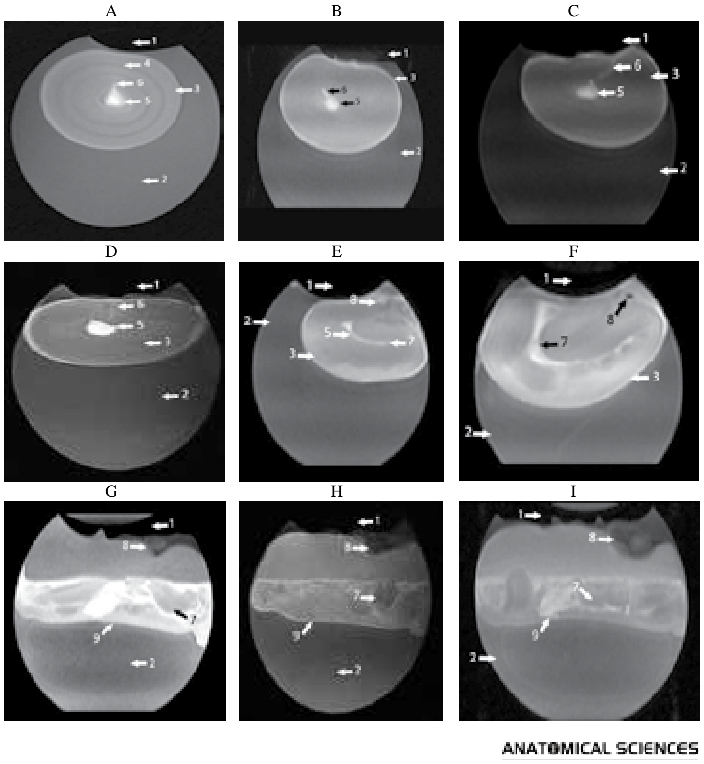

Figure 4. Sagittal MR images from the whole fertilized ostrich egg during the embryonic development. (A) Day 0; (B) Day 2; (C) Day 4; (D) Day 6; (E) Day 8; (F) Day 10; (G) Day 12; (H) Day 16; (I) Day 18: 1-Air cell, 2-Albumen, 3-Yellow yolk, 4-White yolk, 5-Latebra, 6-Neck of latebra, 7-Embryonic vessels, 8-Embryo, 9-Vascular belt

tion within the egg changed noticeably during this work. The yellow and white yolk layers of fertilized egg from day 4 PI were hard and by day 6 impossible to differentiate from each other. The latebra was not observed at day 12 PI onward. In the quail embryo, the disappearing of the yellow and white yolk from the day 4 PI and latebral destruction from the day 6 PI were detected [9]. On the contrary to the quail and ostrich, in the chicken, the latebra is no longer detectable after the yolk rings disappearance [11].

By day 6, blood vessels were visible on the surface of the yolk and the embryo lies close to the top of the egg. The blood vessels provide for absorption of nutrients to the embryo during the growth period [3]. Furthermore, it was suggested that when the vasculature is still forming, it would be advantageous for the embryo to be near the shell to ensure adequate oxygen availability [9]. In chicken model, it has been shown that the vitelline vessels establish yolk circulation in the stages 12 to 16 [3] and yolk absorption begins thereafter. The absorption process definitely needs a time to appear as macroscopic changes in the yolk shape. We did not focused on the differentiation of the vitelline vessels of the ostrich embryo, but based on our results, the shape of the ostrich egg-yolk begins to change from approximately day 6 PI. This change appears as a reduction of the image contrast between different layers of the yolk and finally results in the disappearance of the yolk layers.

In unfertilized eggs, albumen, yellow and white yolk layers, latebra and the neck of the latebra did not change appreciably during incubation. These data suggest that anatomic structures of the unfertilized ostrich egg are quite stable during the first 18 days of incubation period. Moreover, unfertilized eggs images allowed us to have a reference for comparing the consecutive images of the fertilized eggs during this period in order to find out differential changes of the shape and orientation of the egg components.

The MR images of fixed avian embryos have been produced [15, 20, 24, 26] but a few articles have focused on the MRI of live embryos in ovo [6, 9, 10, 11, 17]. As the sample remains intact, MRI is a suitable tool for the time course studies of either normal development or the progression of congenital malformations [14]. The current study has focused upon the MR images of fertilized and unfertilized ostrich eggs and assessment of the developmental changes of the embryo, extra and non-embryonic regions during early stages of development. Our results are in agreement with that of previous studies which concluded that the MRI can be safely used to follow normal development of live avian embryos, in ovo [1, 9].

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