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Morphological development of testes in ostrich (/\textit{Struthio camelus/}) embryo

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Abstract Although the histological structure of ostrich testis has been studied, very little information is currently available on the embryonic development of this organ. The aim of this study was to determine the sequence of the histological changes in diverse components of the testis in ostrich embryo from embryonic day (E) 20 to E42. The main findings were categorized into four histological features, i.e., development of sex cords, interstitial tissue and rete ducts, and the appearance of defective septa. While the lumen of sex cords, tunica albuginea, capsular rete ducts and Leydig cell precursors appeared at E26, the filum-shaped defective septa were visible at E36. The emersion of the lumen in the primary sex cords and formation of capsular rete ducts and Leydig cell precursors appeared at E26, the filament-shaped defective septa were visible at E36. The emersion of the lumen in the primary sex cords and formation of capsular rete ducts and Leydig cell precursors appeared at E26, the filament-shaped defective septa were visible at E36. The emersion of the lumen in the primary sex cords and formation of capsular rete ducts and Leydig cell precursors appeared at E26, the filament-shaped defective septa were visible at E36. The emersion of the lumen in the primary sex cords and formation of capsular rete ducts and Leydig cell precursors appeared at E26, the filament-shaped defective septa were visible at E36. The emersion of the lumen in the primary sex cords and formation of capsular rete ducts and Leydig cell precursors appeared at E26, the filament-shaped defective septa were visible at E36. The emersion of the lumen in the primary sex cords and formation of capsular rete ducts and Leydig cell precursors appeared at E26, the filament-shaped defective septa were visible at E36. The emersion of the lumen in the primary sex cords and formation of capsular rete ducts and Leydig cell precursors appeared at E26, the filament-shaped defective septa were visible at E36. The emersion of the lumen in the primary sex cords and formation of capsular rete ducts and Leydig cell precursors appeared at E26, the filament-shaped defective septa were visible at E36. The emersion of the lumen in the primary sex cords and formation of capsular rete ducts and Leydig cell precursors appeared at E26, the filament-shaped defective septa were visible at E36. Stereological estimations in the ostrich embryo testis showed the major proportion of testis is occupied by the seminiferous tubules, which is unlike the fowl embryo testis.

Keywords Ostrich embryo · Primordial germ cells · Rete ducts · Seminiferous tubules · Tunica albuginea

Introduction

General stages of the testicular development have already been determined in many avian species, and testes in avian embryos are known to arise from the genital ridge at both sides of the dorsal mesentery (Fujimoto et al. 1976; Van Krey 1990). The genital ridges then develop to undifferentiated gonads and subsequently to morphologically identical testes (Smith 2007). During this process, Sertoli cell progenitors and primordial germ cells (PGCs) emerge in the primary sex cords where they become wavy and anastomose to each other, forming the reticular features seen in histological sections (Romanoff 1960; Morish and Sinclair 2002; Gonzalez-Moran and Soria-Castro 2010a). In the fowl embryo, the seminiferous cords lack a lumen by embryonic day (E) 13, but the central axis produces a slit and the lumen appears by E20 (approximate time of hatching) (Gonzalez-Moran and Soria-Castro 2010a). Studies on the morphology and development of testis are mostly performed on mammals and some known birds. To date, the morphological development of testis has been well studied in some avian species, such as domestic fowl and quail (Gonzalez-Moran 1997; Gonzalez-Moran and Soria-Castro 2010a, b; Chang et al. 2012), but pre-hatching studies have been done only on chick and quail testis, with the primary focus on early embryological events in the...
testis (Zaccanti et al. 1990; Sekido and Lovell-Badge 2007; Tagami et al. 2007) and early or late testicular development (Csaba et al. 1980; Shahin and Torok 1982; Gonzalez-Moran and Soria-Castro 2010a, b). There have been few pre-hatching developmental studies in ostrich and other ratite birds, and these have focused mainly on embryonic timing or hatchability of the eggs (Gefen and Ar 2001; Malecki et al. 2005; Nagai et al. 2011). We have recently published a morphological study on the juvenile ostrich testis (Hassanzadeh et al. 2013), and in a few other studies the authors have explained the morphological features of adult ostrich testis (Soley 1992; Aire and Soley 2003; Ozegbe et al. 2006, 2008, 2010; Lan et al. 2007; Zhang et al. 2011); however, developmental studies of ostrich testis are still scant and limited to the post-hatching period (Madekurozwa et al. 2002; Wei et al. 2011). Budras and Meier (1981) have performed the only developmental study on the testicular excrurrent ducts in ratite birds. This study covers both the pre- and post-hatching periods, and the testis in the juvenile and adult ostrich was compared with that in rhea and emu. 

In the study reported here, we studied in detail the morphological development of ostrich testis, including the formation of sex cords, testicular capsule and capsular rete ducts. We also evaluated the morphometrical changes in the testis and its tubular compartments quantitatively using stereological techniques. Our results provide novel information on the developmental characteristics of the ostrich testis.

Materials and methods

Animals and tissue samples

A total of 50 fertile ostrich eggs obtained from commercial farms were incubated at 36–37 °C and 25 ± 2 % relative humidity with tilting to 90° at 4-h intervals for 20, 26, 36 and 42 days. Due to the coincidence of E42 with hatching day and in order to minimize the number of animals sacrificed, hatched chicks were sexed using the method of Malago et al. (2002). After decapitation of male chicks and embryos and the drainage of blood, the testicles were anatomically analyzed, photographed and separated from the body and placed in Bouin’s solution. Three samples of testes at each of E26, 36 and 42 (size approx. 1 mm³) were taken and placed in 2 % glutaraldehyde in 1 M cacodylate buffer for embedding in resin. All procedures were approved by the Ethics Scientific Committee of the Ferdowsi University of Mashhad.

Morphometry

Stereological techniques were employed to determine the amount of testicular volume occupied by seminiferous tubules (volume fraction or volume density of seminiferous tubules in the testis). After fixation, samples (whole testes) were dehydrated in increasing degrees of ethanol, cleared in xylene and embedded in paraffin. Due to the existence of a directional asymmetry (Moller 1994), the stereological experiments were done only on the left testicles. Tissue shrinkage was calculated by measuring testicle diameter just before fixation and embedding in paraffin. Paraffin-embedded samples were cut into 5-μm-thick serial sections and sampled (at least 11 sections per sample) according to the unbiased systematic random sampling (SRS) method. After staining of the sections with Periodic acid–Schiff (PAS) stain, several fields from large sections were selected and imaged according to SRS method. The images were placed under a stereology grid (with 300 intersection points without any direction), and when the points were positioned on the whole section, the reference area and seminiferous tubules were analyzed and counted. The total volume of the testis (Tes.V) was estimated according to the Cavalieri’s principle. The volume fraction of seminiferous tubules in each testis (Vv) was calculated using Eqs. 1, 2 and 3 based on the Delesse principle (Delesse 1848; Howard and Reed 2005):

\[
Vv = \frac{\bar{a}}{A} \quad (1)
\]

\[
\bar{a} = \frac{a_1 + a_2 + \cdots + a_k}{k} \quad (2)
\]

\[
A = \frac{A_1 + A_2 + \cdots + A_k}{k} \quad (3)
\]

Where \( a \), \( A \) and \( k \) are the area occupied by seminiferous tubules, the reference area and the number of images, respectively. The volume of testis was multiplied by Vv to estimate the total volume of seminiferous tubules (S.T.V). The results were analyzed by one-way analysis of variance, and the different groups were determined by Tukey’s multiple comparison test. Differences among mean Vv of the seminiferous tubules were considered to be statistically significant at \( P < 0.05 \).

Histology

The glutaraldehyde-fixed samples were re-fixed in 1 % osmium tetroxide in 1 M cacodylate buffer. These samples were dehydrated by the progressive lowering temperature (PLT) method, embedded in epoxy resin (TAAB Laboratories Equipment Ltd, UK) and cut in 1-μm-thick sections. The sections were stained with toluidine blue in order to identify the lumen of the sex tubules and the lipid granules of the Leydig cells (Kuo 2007). Some of the 1-μm-thick sections from different parts of the right and left testes, near two poles and the equatorial border, were selected for staining with PAS, hematoxylin and eosin (H&E), Mason’s trichrome (MT) and Alcian blue (AB) to clarify the
details of seminiferous tubules, interstitial tissue and the testicular capsule. PGCs were recognized based on their morphological characteristics, which have been described previously (Ginsburg and Eyal-Giladi 1986; Ishiguru et al. 2009; Gonzalez-Moran and Soria-Castro 2010a; Wei et al. 2011). Images were collected on a digital camera (model SX210 IS; Canon Inc., Tokyo, Japan) and using a light microscope (models BX51 and 60; Olympus, Tokyo, Japan) equipped with a digital camera (model DP12; Olympus). The acquired images were prepared by Image J (National Institutes of Health, Bethesda, MD), Paint (Microsoft, Redwood, WA), and Snagit Editor (TechSmith Corp., Okemos, MI) software.

Results

Gross anatomical changes

In the 20-day-old embryo, testes having a dirty-white color were located on the ventral surface of mesonephros kidneys. The testes extended from the cranial edge to the midportion of kidneys, where they inclined toward the median plane of body and were located close to each other. At this stage, the testes had a curved tortuous or elongated ovoid shape and were directly attached to the kidneys (Fig. 1a).

In the 26-day-old embryo, the testes had become brighter and were clearly distinguishable from the kidneys in texture and color. Their dimensions had increased and the connection to the kidney had become narrower and distinct. Their shape changed to elongated ovoid and became regular relative to the previous stage (Fig. 1b). In the 36-day-old embryo, the testes had become larger and due to the regression of mesonephros and growth of metanephros their location changed caudally toward the metanephros middle lobe (Fig. 1c). In the 42-day-old embryo or newly hatched ostrich, the testes extended from the mid-portion of the cranial lobe of the metanephros to its caudal edge (Fig. 1d). In all cases the longitudinal axis of testes made an acute angle with the sagittal axis of the body which was greater on the right side. The shapes of the testicular transverse sections changed from elongated in the 20-day-old embryo to bean-shaped in the 26-day-old embryo, and then to ovoid in the 36-day old embryo and subsequently to a circular shape in the 42-day-old embryo.

Morphometrical changes

Stereological techniques were used for evaluating morphological changes in the testis, including the Tes.V, Vv of the seminiferous tubules in the testis, and S.T.V. in each testis. Evaluations showed that body weight (B.W.) increased from E20 to E36 and thereafter slightly decreased up to the hatching day (Fig. 2a). The Tes.V. and S.T.V. increased linearly up to the hatching day (Fig. 2a). In general, the ratio of Tes.V. and S.T.V. to the BW decreased from E20 to E42 (Fig. 2b). The estimated Vv decreased from E20 to E26 but increased slowly up to E42 (Fig. 2c).

Histological changes

The 20-day-old embryo

Sertoli cell precursors surrounding the PGCs formed Sertoli cell aggregations which extended toward each other and in some cases came into contact and anastomosed (Fig. 3). PGCs were distinguishable by their relatively large, pale, spherically shaped nucleus (Fig. 3a, c). In MT- and AB-stained sections, the nucleus of mitotic PGCs appeared as a dark flocculus of chromatin (Fig. 3b, d). Sertoli cell precursors had elongated ovoid or triangular nuclei which were smaller and more heterochromatic than the PGC nuclei, whereas when treated with AB stain the nuclei of the former stained pale relative to those of the PGCs (Fig. 3). The Sertoli cell precursors had a polar epithelial...
cell-like morphology with numerous secretary granules at the apical pole and the nucleus at the basal pole. The apical pole was more ramshackle than the basal pole which was supported by a delicate basement membrane (Fig. 3c). Interstitial tissue contained only PAS-positive fibers that appeared pink or purple in color, while Leydig cell precursors were undistinguishable from mesenchymal cells (Fig. 3). Testicular capsules consisted of a single layer of cuboidal cells as a covering epithelium and its underlying blood vessels (Fig. 3b). Attachment to the kidney was devoid of covering epithelium, where a few rete ducts paved by flattened cells were present (Fig. 3a).

**Fig. 3** Sertoli cell aggregations (some of them defined by broken lines) in the 20-day-old ostrich embryo. a Illustration of the rete ducts (R) paved by flattened cells at the attachment site of testis and kidney. Note the differences between the rete ducts and blood vessels (V). Hematoxylin and eosin (H&E) staining. b Sertoli cell aggregations came into contact and anastomosed to each other. Note the anastomosing site (asterisks), testicular covering epithelium (Ep) and its underlying blood vessels (V). Masson’s trichrome (MT) staining. c The primordial germ cell (PGCs, P) with a large, pale, spherical nucleus are located between the Sertoli cells (Sr). The color of interstitial tissue (I) is because of its Periodic acid–Schiff (PAS)-positive fibers. Arrows Basal pole of Sertoli cells closed to the basement membrane, arrowheads apical pole. PAS staining. d Interstitial tissue fibers were distinctly stained by Alcian blue (AB) while the nuclei of the PGCs stained as dark flocci. Ms mesonephric tubule, Bg blood globule. Scale bar 30 µm

Elongating and anastomosing cords superseded Sertoli cell aggregations causing a net-like structure (Fig. 4b, c) to develop. The number of PGCs was clearly increased relative to E20, but their morphology was the same except for their completely eccentric location in the cords (Fig. 4a, b).
The central portion of the cords had a different texture from the other parts, and a PAS-positive line had circled this portion which made it look like a lumen (Fig. 4c). Existence of this lumen was completely distinct in resin sections (Fig. 5a). A few Leydig cell precursors were distinguishable based on their lipid granules (Fig. 6a). Two types of connective tissue fibers, namely, red and green fibers, were distinct only in the MT-stained sections (Fig. 4b). The tunica albuginea appeared between the covering epithelium and its underlying blood vessels, causing increasing thickness of the capsule (Fig. 4b). In MT-stained sections, similar to the interstitial tissue, fibers were mostly red and only rarely green, (Fig. 4b). The thickness of this layer at the cranial and caudal pole of testis was more than that at the equatorial area. Some cell aggregations similar to Sertoli cells were trapped in the tunica albuginea, but a number of larger cells with the morphology of PGCs were also present (Fig. 4d). These aggregations were transforming into a tubule or duct, and in some areas they were located inside the larger cisterns (Fig. 4d). The marginal blood vessels were following a distinct model which was clear only in the sections of the equatorial part of the testis (Fig. 4c). In the hilar portion, the primary sex tubules were directed toward the hilus and terminated in the lacunar rete ducts (Fig. 4d).

**Fig. 4** Network of anastomosing primary sex cords, tunica albuginea and capsular rete appear in 26-day-old ostrich embryo. Transverse sections from the cranial pole (a), caudal pole (d) and equatorial area (b, c) of the testis. a Cell aggregations (black arrowheads) trapped in the capsule containing mostly Sertoli-like (Sl) cells and only rarely PGC-like (Pl) cells. H&E staining. b Anastomosing primary sex tubules (broken lines) formed a net-like appearance. Remnants (H) remained after loss of the tubules lumen (asterisks) contents. MT staining. c Capsular blood vessel canalization; longitudinal vessels (VI) connected by transverse vessels (Vt). PAS-positive lines (Ps) define the boundary of lumens. PAS staining. d In some areas capsular cell aggregations (black arrowheads) were located in the larger cisterns (arrows). The magnified images show their transformation to rete ducts (d') and morphological details of PGCs (d''). AB staining. Ep Covering epithelium, P PGC, Pm mitotic PGC, Sr Sertoli cell, Ca capsule, I interstitial tissue, R lacunar rete ducts, V blood vessel, Ms mesonephric tubule, Mg mesonephric glomerulus. White arrowhead Basement membrane. Scale bar 50 μm

The 36-day-old embryo

The primary sex cords became wavy and formed a net-like structure. The PGCs had the same morphology, but they had...
increased in number, and most of them were mitotic. Leydig cell precursors were widespread throughout the interstitial tissue. The polymorphic nucleus of these cells, as well as the presence of a few lipid granules, distinguished them from the fibroblasts and blood cells (Fig. 6b). The testicular capsule, which completely coated the testis, had acquired a

Fig. 5 Appearance of lumen in the primary sex cords. Transverse semithin sections of the ostrich testis stained by toluidine blue indicate the presence of a lumen (arrows) in the primary sex cords on E26 (a), E36 (b) and E42 (c). Note that the apical pole of Sertoli cells contains numerous secretory granules and that the basal pole usually contains the nucleus. d Circling fibroblasts (F), around the seminiferous cords, which are presumably the progenitors of the myoid cells. Ca Testicular capsule, I interstitial tissue. Asterisks PGCs, broken lines boundary of sex cords. Scale bar 10 μm

Fig. 6 Appearance of Leydig cell lipid granules. Transverse semithin sections of ostrich testis stained by toluidine blue shows the presence of lipid granules (arrowheads) on E26 (a), E36 (b) and E42 (c). Periphery of sex cords (S) are marked by broken lines. Bl Blood cells, I interstitial tissue. Asterisks PGCs. Scale bar 10 μm
tree-partite structure that included the tunica serosa, tunica albuginea and tunica vasculosa (Fig. 7a, b). Extending from the tunica albuginea, some meandering rays penetrated the interstitial tissue (I). In the central part of the testis some arteries can be seen to be embedded inside the defective septa (b').

c Distribution of capsular rete (arrowheads) and large cisterns (arrows) in the testicular free surface and their histological details (c' and c''). Note the presence of PGC-like (Pl) and Sertoli-like (Sl) cells in the structure of capsular rete ducts, and the cuboidal epithelium (Co) of the large cisterns. d Histological details of capsular cell aggregation (arrowheads) which still had not acquired a duct-like shape (d''). Note the typical structure of a muscular artery (d''') including the tunica intima (In), tunica media (Md) and a fibrous tunica adventitia (Ad) which merged with the fibrous texture of their container defective septum. Bg Blood globule, Ca testicular capsule, P PGC, Pm mitotic PGC, S seminiferous tubule, Sr Sertoli cell, Ts tunica serosa, Tv tunica vasculosa, V blood vessel in tunica vasculosa. Asterisk Lumen of seminiferous tubules, arrows large cisterns, arrowheads capsular rete ducts. Scale bar 50 μm

The 42-day-old embryo

In the chick embryo, the primary sex cords became wavier and formed a reticular structure while the confines of their lumen became more distinct (Fig. 5c). It appeared that the number of PGCs had not increased relative to E36. The tree-partite structure of the capsule had become more distinct and completely coated the testis (Fig. 8a). The capsular rete ducts had expanded toward the free surface of the testis (Fig. 8a, b) and the thickness of the capsule was increased due to intensification of fibers in the tunica
albuginea, while the inner vasculosa and outer serosa layers were approximately constant (Fig. 8a). More and larger lipid granules were found in the cytoplasm of Leydig cell precursors (Fig. 6c). There were no morphologically distinct peritubular myoid cells, but some fibroblasts were clearly circling around the seminiferous tubules (Fig. 5d).

Discussion

The main findings of this study were categorized into the development of four histological features: (1) the development of sex cords; (2) the development of interstitial tissue; (3) the development of rete ducts; 4) the appearance of defective septa. A general observation was that the development of sex cords between E20 and E42 in the ostrich was similar to that reported in other birds. Our results indicate that the primary sex cords in ostrich originate from Sertoli cell aggregations which elongate, contact and anastomose with each other during embryonic development, similar to what occurs in other birds. Whereas the formation of primary sex cords in fowl was completed by E7.5 of the first half of the embryonic period (Morrish and Sinclair 2002), in the ostrich embryo primary sex cord formation was completed at E26 of the second half of the embryonic period. According to the previous reports, the appearance of a lumen in the primary sex cords is postponed until hatch in birds and until puberty in mammals (Romanoff 1960; Skinner and Griswold 2005; McGeady et al. 2006; Gonzalez-Moran and Soria-Castro 2010a; Sadler 2010), but in the ostrich embryo the lumen began to form on E26, and on E36 a distinct lumen was present; consequently, these cords can be called “primary sex tubules”. One important feature of these cords is that they contain filled lumens. Resin sections, but not paraffin sections, demonstrated that the lumens were filled with a semisolid fluid. Furthermore, the unoccupied spaces usually seen in the lumen of post-embryonic seminiferous tubules were also due to the absence of its fluid contents (Hassanzadeh et al. 2013).

The most important finding regarding the development of the interstitial tissue was that fibroblast-like Leydig cells appeared on E26. In ostrich testis, the appearance of fibroblast-like Leydig cells began from E26 onwards, while in fowl this event occurs at E8 (Gonzalez-Moran and Soria-Castro 2010b) in the first half of the embryonic period. The presence of these cells has been reported in the testis of Japanese quail (Nicholls and Graham 1972) and 2-day-old chicken (Connell 1972), as well as in mammals (Christensen and Fawcett 1961; Black and Christensen 1969), and they transform from a fibroblast-like form, through a transitional stage, to a mature androgen-producing Leydig cell form (Narbaitz and Adler 1966; Connell 1972; Jordanov et al. 1978; DeFalco et al. 2011). The presence of lipid granules, which were dark in coloration in most of our images, is an important characteristic by which to identify these cells. This is in contrast to the white coloration of these granules that has been reported by other authors (Nicholls and Graham 1972; Gonzalez-Moran and Soria-Castro 2010b). This difference might be related to the specific methodology used in the study. The PLT method used in our study preserves the lipid contents of Leydig cell granules, which are then stained by osmium tetroxide. It was not possible to define the exact boundaries of Leydig

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**Fig. 8** Detailed histological characteristics of testis on E42. Transverse sections stained by MT (a) and H&E (d). a Presence of developing capsular rete ducts (arrowheads) at the free surface of the testis beside a defective septum (Df) are shown (a’ and a”). Note that the central portion of seminiferous tubules and rete ducts have been lost during the staining procedure. b Developing capsular rete ducts (arrowheads), containing PGC-like (Pl) and Sertoli-like (Sl) cells in the lateral surface of testis are still present. The magnified parts (b’ and b”) show their morphology in details. H Hilus of testis, I interstitial tissue, P PGC, S seminiferous tubule, Ta tunica albuginea, Ts tunica serosa, Tv tunica vasculosa. Arrows Large cisterns, arrowheads capsular rete ducts. Scale bar 50 μm.
cell precursors due to the compression of cells and the randomness of the fibers in the interstitial tissue. At first, the fibers of the interstitial tissue were not distinct collagen fibers (Eurell and Van Sickle 2006), but during development collagen fibers appeared, increased in number, and finally caused the change in interstitial tissue color from red to a mixed color of green and red. At the territorial portions of the interstitial tissue, a group of interstitial tissue fibroblasts similar to peritubular myoid cells were observed encircling the seminiferous tubules. These cells, however, did not have the distinct morphological characteristics of the myoid cells which are seen in the juvenile ostrich testis (Hassanzadeh et al. 2013). It might be possible that these encircling fibroblasts acquire the morphology of myoid cells during post-hatching developmental processes.

Rete ducts are other noticeable structures of ostrich testis that consist of three parts of intratesticular, extratesticular or lacunar, and intracapsular rete ducts or true rete with large cisterns (Budras and Meier 1981; Aire and Soley 2003). We recently investigated the capsular rete, the important part of this system, and reported its extension up to the free surface of the testis in the juvenile ostrich (Hassanzadeh et al. 2013). In accordance with that report, our results also show that the true rete is present in the medial, lateral and free surfaces of the testis from the prehatching period. Since rete ducts were frequently seen in the capsule of the polar parts, we can conclude that these ducts might first appear in the polar parts of the testis and subsequently extend toward the equatorial parts. Budras and Meier (1981) proposed that the buds of glomerular capsules of mesonephros are the origin of rete testis, but our results indicate that the capsular rete develops from the cell aggregations trapped inside the tunica albuginea which are comprised of two cell types: Sertoli-like and PGC-like cells. Thus, it is a plausible speculation that the capsular rete ducts may originate from the primary sex cords. Despite the presence of primary sex cords inside the testis, the thickness of the epithelium in these capsular aggregations decreases during embryo development and ultimately forms a squamous or flattened cuboidal epithelium (Hassanzadeh et al. 2013). However, regarding the structural differences between different parts of the rete system (Budras and Meier 1981; Aire 1982; Barker and Kendall 1984; Aire and Soley 2003), it is possible that its different parts develop from diverse origins.

As in other birds, in the ostrich the covering epithelium and its underlying blood vessels are present from the early stages of testis formation, while its third layer, the tunica albuginea, appears later (Gonzalez-Moran and Soria-Castro 2010a). It has been shown that tunica albuginea, the main part of the testicular capsule, is considerably different in the ostrich (Ozegbe et al. 2008; Hassanzadeh et al. 2013). In the ostrich embryo, this layer first consists of a loose connective tissue, but due to intensification of collagen fibers (which stain green by MT), changes to a dense connective tissue in the newly hatched ostrich (Nicholls and Graham 1972; Eurell and Van Sickle 2006). Initially this layer had a loose structure, and its internal components, such as the capsular rete ducts and blood vessels, appeared in their real shapes in the sections. After densification, which forces these structures to collapse, it was difficult to find these internal components in the sections. Hardening and thickening of the tunica albuginea has another outcome, i.e., the appearance of defective septa. In mammals, testicular septa branch from the inner part of the capsule and conduct blood vessels and nerves into and out of the testicular substance (Davis et al. 1970). In earlier studies, it was believed that these septa were absent in birds (Lake 1971; Hodges 1974), but their presence were subsequently reported in ostrich (Soley and Groenewald 1999; Hassanzadeh et al. 2013). Our observations confirm the presence of defective septa and suggest that the mammalian lamellar septa are the evolutionary remnants of the defective septa in ostrich which have a filum-like shape. The defective septa penetrated the interstitial tissue in a meandering way while conducting blood vessels and nerves into and out of the testis.

While classical measurements are routinely used in anatomical and embryological studies, new methods such as stereology have been applied recently in studies on avian developmental biology (Gonzalez-Moran 1997, 2011; Gonzalez-Moran and Soria-Castro 2010a, b). In addition to measurements of B.W. and testis diameter, we used stereological techniques to estimate the Tes.V and Vv of seminiferous tubules. The weight of embryos increased in a linear manner during the period leading up to the hatching day, but it decreased during the day of hatching (Fig. 2a). This is a common finding in newborns in both mammals and birds (Mortola 2009; Eidelman et al. 2012). Our results show that testis and seminiferous tubule volumes increased during the embryonic period and that this pattern of increase was similar to that of the respective growth curves. Thus, the ratios of Tes.V/B.W. and S.T.V/B.W. fell on E26 (Fig. 2b). Considering that the Vv of seminiferous tubules generally decreases from E20 to E42 and reaches about 0.56 in the juvenile ostrich (Hassanzadeh et al. 2013), it is possible to conclude that there is no direct relationship between the B.W. increase and changes in the Vv of tubular compartments. However, a decrease in Vv between E20 and E26 (Fig. 2c) could be related to histological changes in the components of the testis other than seminiferous tubules, including changes in the testis capsule (appearance of tunica albuginea) and the development of new blood vessels. In comparison with other animals, increased Vv of seminiferous tubules from prenatal to
pre-pubertal period is very different from the increasing pattern in domestic fowls and bonnet monkeys (Macaca radiata) reported previously (Gonzalez-Moran and Soria-Castro 2010a; Prakash et al. 2008).

In summary, we report here developmental changes in the testis of the ostrich embryo based on histological and morphometrical results and highlight differences in the development of capsular rete ducts, defective septa and changes in the Vv of tubular compartments in testis of ostrich embryo in comparison with other birds. Our results provide new information on the appearance of the lumen in the primary sex cords, which is unique to the ostrich among birds and mammals.

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Conflict of interest None.

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