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Comparative Histologic Evaluation of Vertebrate Ovaries

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Abstract. Comparative Anatomy is a subspecialty of Anatomical Sciences that deals with/involves the possible existing structural relatedness in organisms. By careful description of specific structures, organs or systems, comparisons are made that draw similarities or differences between organs. The aim of this area in Anatomy was to provide evolutionary or adaptive explanations for detected characteristics – including maintaining the ancestral line.

Materials and Methods

Permission for this descriptive and observational study was obtained from the research and ethics committee of the Department of Human Anatomy and Cell Biology, Delta State University, Abraka (Number DELSU/CHS/ANA/118). Five female animals of the vertebrate class were used for the study (one from each vertebrate class). The animals included: cat fish (*Clariasgariepinus*); toad (*Bufobufo*); agama lizard (*Agama agama*); domestic chicken (*Gallus gallusdomesticus*) and the Wistar rat (*Rattusnorvegicus*).

Results

All the studied vertebrates except mammals showed growth and maturation of several follicles confirming the characteristic of multiple gestation characteristic of these animals. The phases of growth were very similar in all of the studied organisms. These follicles were disposed in a fibrous stroma capable of influencing the activity of the ovary, Lacunae were only found in birds and mammals.

Conclusion

The index study revealed several significant findings especially the occurrence of multiple stages of germ cell development in the female vertebrate gonads. These observations provided a vivid histologic basis for the argument of a common ancestral origin of the animal phylum albeit against the background of extensive impact of adaptational factors.

Keywords: Comparative; Histologic; Vertebrates; Female; Gonads; Evolution; Adaptation.

Introduction

The discipline of Comparative Anatomy involves provision of detailed, albeit; elaborate background facts of the structural relationships between and within organisms. By careful description of specific structures, organs/systems, comparisons are made that generate specific characteristics required for demonstrable variations in organisms. The aim of this interesting discipline is to provide evidence of the role of evolution or/and adaptation in formation of the determined features of organisms.

Reproduction is one of the characteristics of living things. This process, whose aimed principally at production of offspring, is proficient in the animal phylum.[1] The phylum, composed of both non-vertebrates and vertebrates is responsible for a major component of the animal kingdom. It had been opined in a previous inquiry that propagation of vertebrate species depended on the degree of development of the reproductive apparatus which were vital for the support of germ cells critical in gonadogenesis.[2]

The emphasis in the index study on vertebrates was the female gonads and with basic histologic techniques, fascinating explanations for the designs of the reproductive apparatuses in the vertebrate class from observed architectural similarities and available differences in the ovaries, were provided. Ultimately, the relatedness (phylogeny) was established in the five (one species per class) vertebrate classes.

Materials and Methods

Permission for this comparative histologic experimental study was sought and received from the research and ethics committee of the Department of Human Anatomy and Cell Biology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka (Number DELSU/CHS/ANA/118). The research was conducted in accordance with the guidelines for the care and use of animals for research.[3-4]

Five animals were utilised for the study (one from each vertebrate class). The animals included: common cat fish (*Clariasgariepinus* – fish); the toad (*Bufobufo* - amphibian); the agama lizard (*Agama agama* – reptile); the house hold chicken (*Gallus gallusdomesticus* – bird) and the Wistar rat (*Rattusnorvegicus* – mammal).

Following euthanasia, the ovaries of each animal were dissected out and preserved in 10% formal saline for about 48 hours. The samples were then processed using standard procedures and stained with routine histological stains (haematoxylin and eosin).[5] The histology slides obtained were then analysed and interpreted with the aid of a Scopetek Digital Camera Microscope (DCM) 500, 5.0 mega pixel attached to a computer and the obtained results then were displayed in figures and tables.

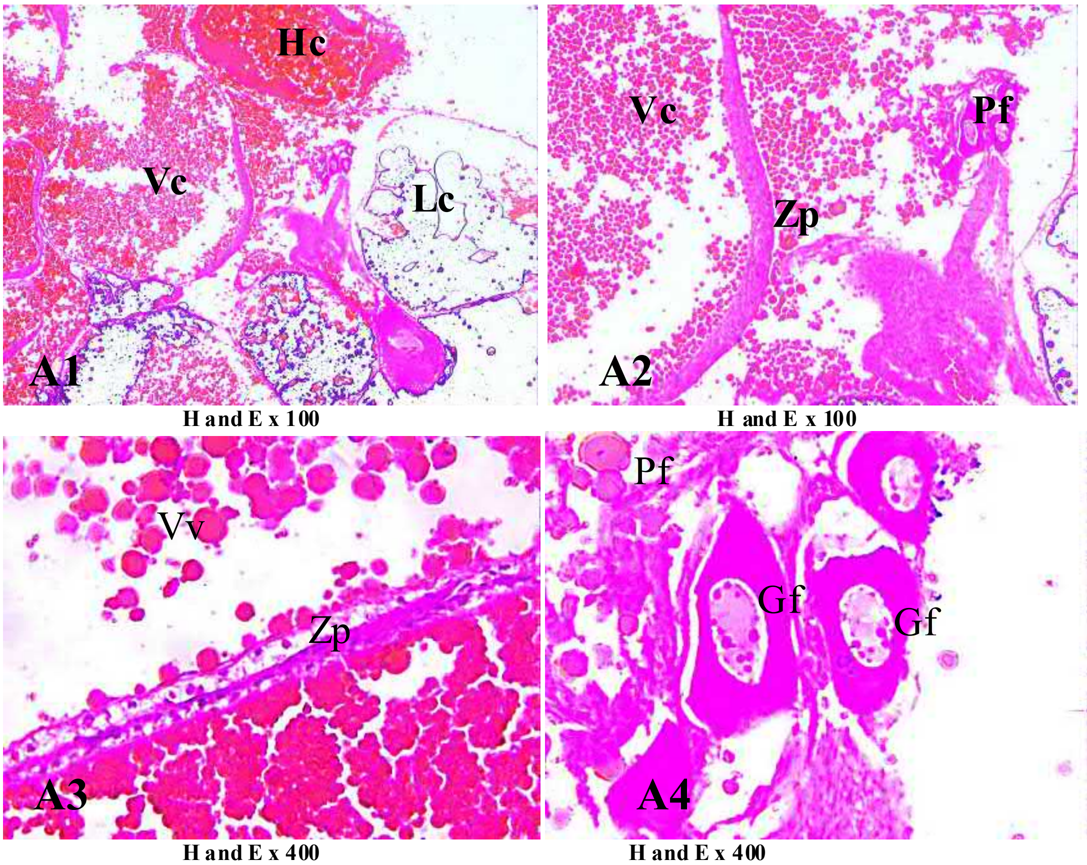
Micrographs **A1 to A4** are sections of cat fish ovaries showing several variably sized follicles disposed in clusters with each composed of oocytes **(A1 and A2)** enclosed by a dense connective tissue–zonula pellucida and a two layered follicular cell covering as shown in table 1 and **(A3)**. Within the cytoplasm of each oocyteare abundant round to oval shaped deeply eosinophilic vesicles apparently blunting the silhouette of other cytoplasmic organelles **(A3)**. A centrally disposed round to oval but bland nucleus, with multiple nucleoli in their nucleoplasm, is prominent in younger follicles **(A4)**.

As displayed in micrographs **B1-B4**, sections through the female gonads in a toad show several variably sized ova composed of abundant basophilic and small round to oval sized vesicles enclosed by a thin walled layer of flattened epithelium resting on a connective tissue stroma (**B1**). A centrally placed pale variably sized nucleus is present in each ovum **(B2 and B3)**. Each follicle is lined by a layer of flattened epithelium resting on a conspicuous dense regular connective tissue stroma (table 1) which encloses a cytoplasm containing granules displaying intense eosinophilia (**B4**).

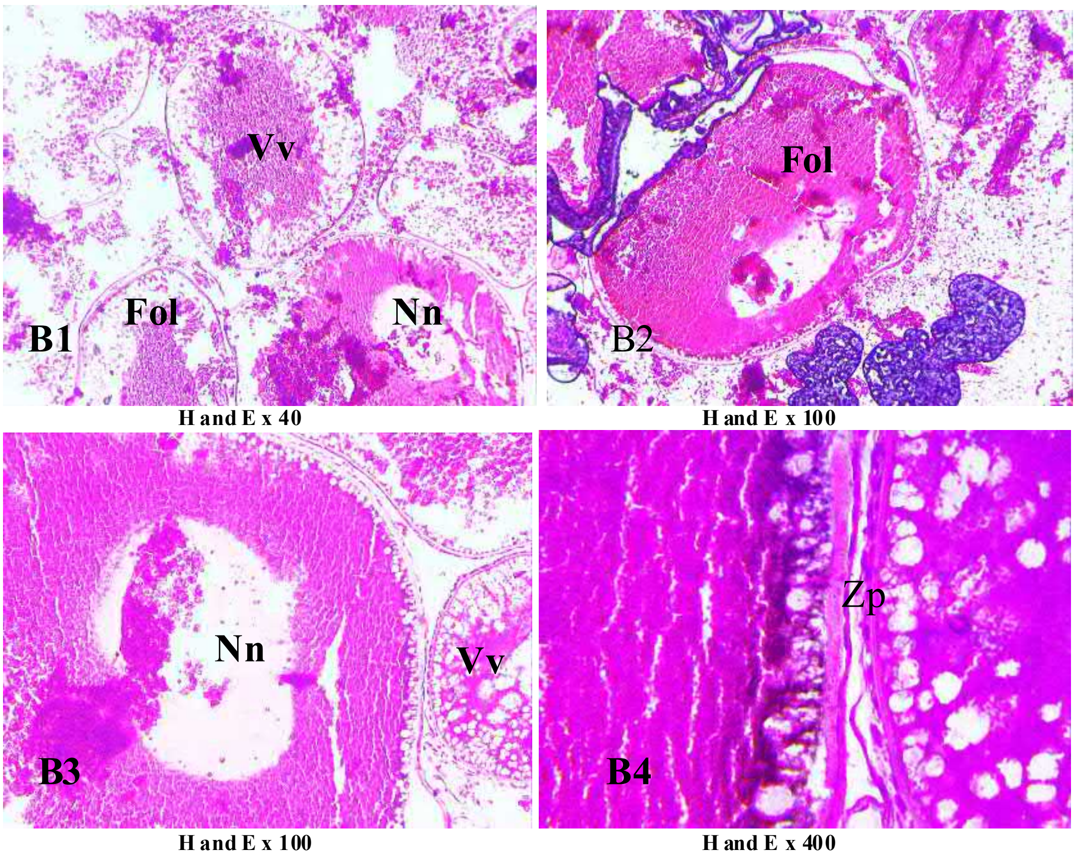
The ovaries of lizards, micrographs **C1** to **C4**, reveal few follicles at different stages of maturation disposed in a thin connective tissue stroma. Immature follicles composed of a mononucleated ova enclosed by a single layer of flattened to cuboidal cells with centrally placed nuclei and pale amphophilic cytoplasm are also seen, **C1 and C2**. There is an obvious nucleus in some of these follicles while the multiple fine granules all enclosed within a multi-layered zone of pale cells with bland basophilic cytoplasm, is prominent in others (**C3 and C4**). Although mildly obscure, lacunae and thin walled blood vessels are also seen in the medullary portion (table 1) of the ovary.

Bird ovarian tissue show several follicles at different stages of development disposed in a fibro myxoid connective tissue stroma (**Micrograph D1 to D4**). The follicles range from primordial, primary to secondary follicles **(D1-D2)**. Ova are observed topossess a nucleus displaced towards the periphery of the cell amidst a myxoid cytoplasm all enclosed by a dense pellucidal zone **(D3-D4)**. Lacunae and thin walled blood vessels are also seen in the medulla. Also seen are luteal corpora, corporal atreticum and corpora albicanthes disposed within the ovarian stroma.

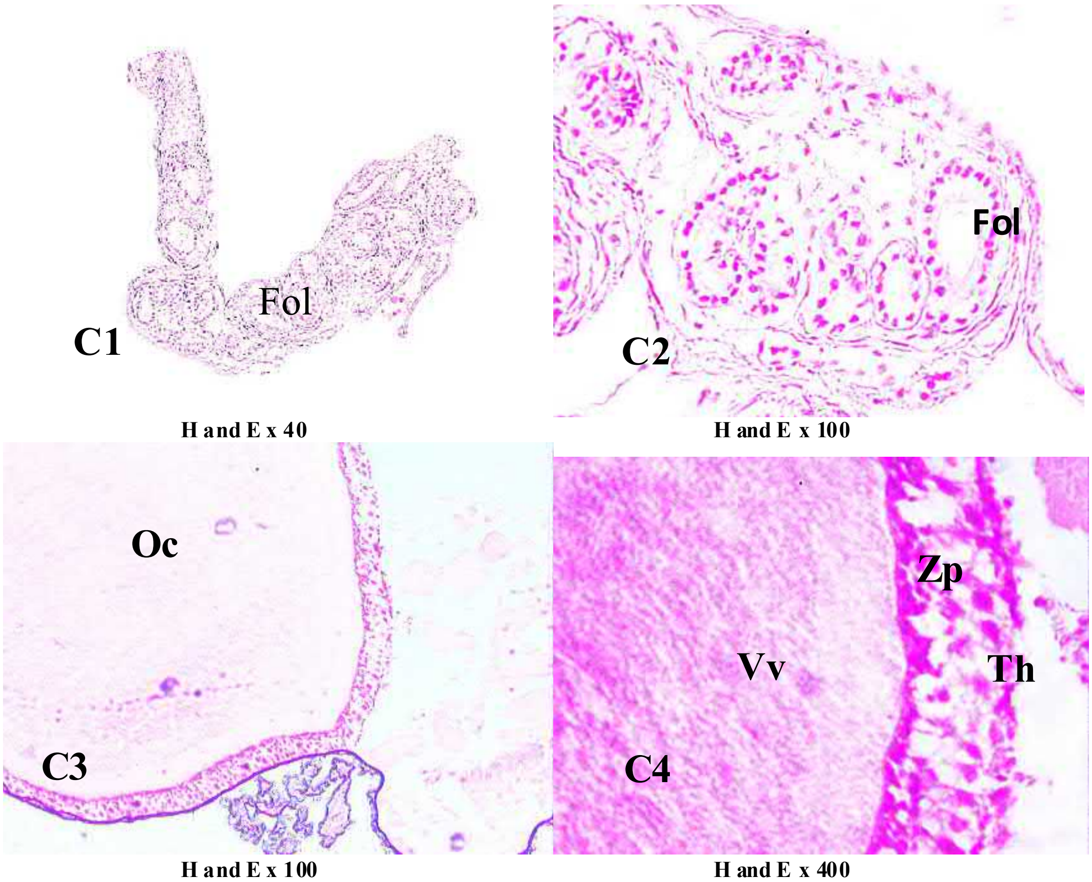
In rats, micrograph **E1** to **E4**, the ovaries are composed of severally variably sized follicles separated by an irregular dense fibro-connective



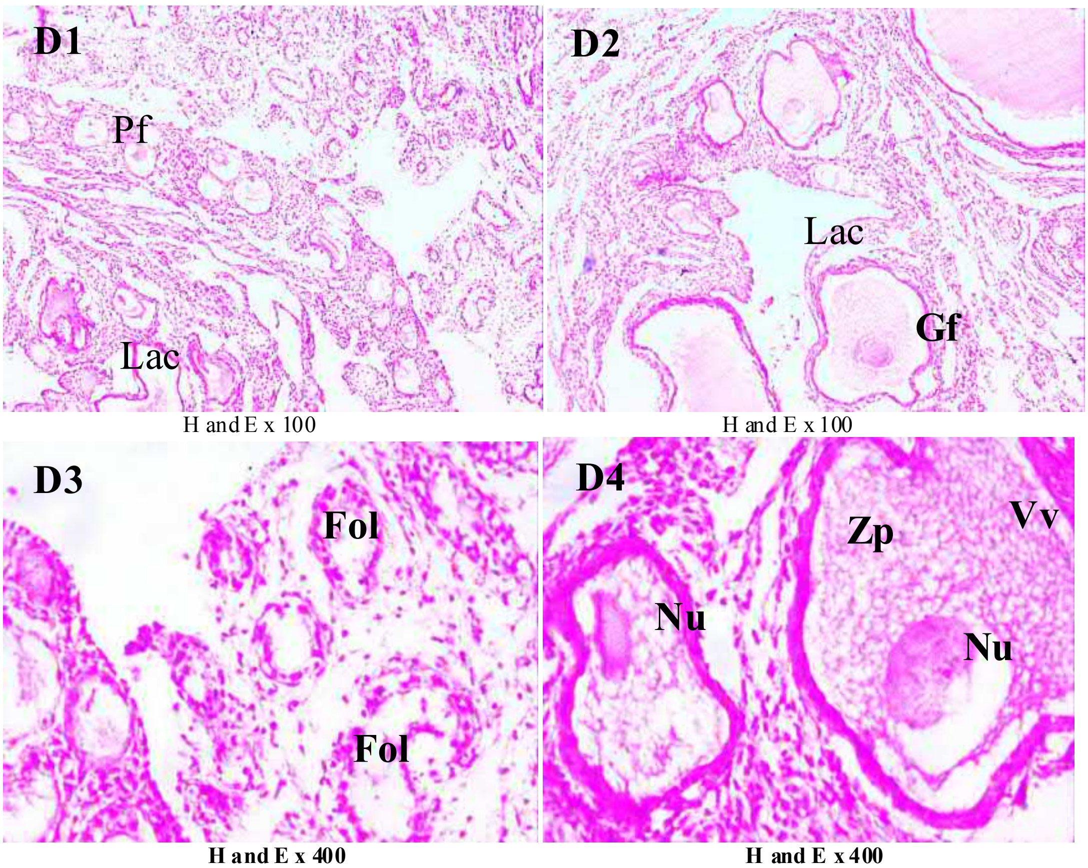
Micrograph A1: Section of Fish ovary showing **vesicles cytoplasm(Vc), Haemorhagic corpus (Hc) and Lacunae (Lc); MicrographA2**: Section of fish ovary showing **Zonula pellucidum (Zp), vesicular cytoplasm(Vc), Primary follicles(Pf); MicrographA3:** Section of fish ovary showing **zonula pellucidum, theca (ZT**) and**Vesicles (Vv); MicrographA4:** Section of fish ovary showing **primary follicles (Pf), Growing follicles (Gf)**



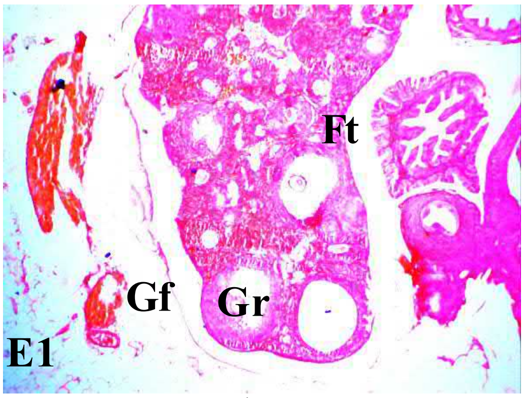
Micrograph B 1: Section of toad ovary showing a **Follicle (Fol), vesicle (Vv) and nucleus (Nn); Micrograph B2**: Section of toad ovary showing a **Follicle(Fol); Micrograph B3**: Section of toad ovary showing **Nucleus (Nn)** and **Vesicles (Vv); Micrograph B4**: Section of toad ovary showing **Zona pellucida (Zp)**



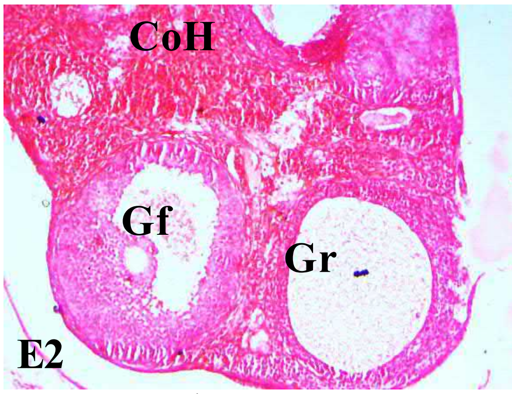
Micrograph C1: Section of lizard ovary showing a **Follicle (Fol);Micrograph C2:** Section of lizard ovary showing a **follicle (Fol); Micrograph C3:**Section of lizard ovary showing an **Oocyte (Oo); Micrograph C4:**Section of lizard ovary showing **vesicles (Vv), Theca (Th)** and **Zona pellucida (Zp)**



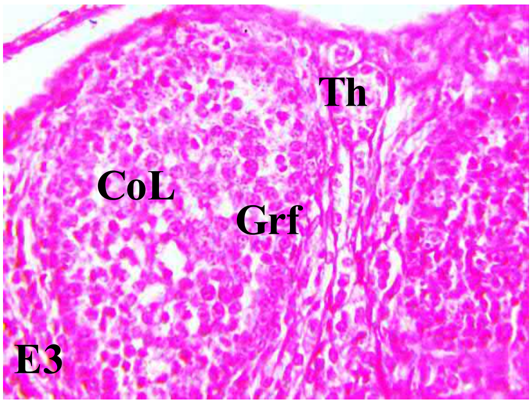
Micrograph D1: Section of bird ovary showing a **Primordial follicle (PF)** and **Lacunae (Lac); Micrograph D2:** Section of bird ovary showing a **Lacunae (Lac)**and**Graafian follicle (Gf); Micrograph D3:** Section of bird ovary showing an follicle **(Fol); Micrograph D4;** Section of bird ovary showing **vesicles (Vv), Nucleus (Nu)** and **Zona pellucida (Zp)**



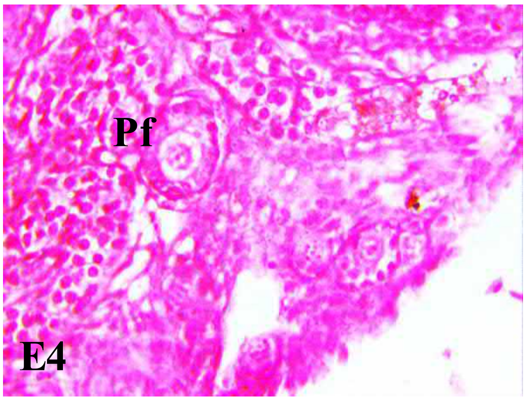
H and E x 40



H and E x 100



H and E x 400



H and E x 400

Micrograph E1: Section of rat ovary showing a fallopian tube (Ft) andGraffian follicle (Gr); **Micrograph E2:** Section of rat ovary showing Graafian follicle (Gr) and Corpus Haemorrhagicum; **MicrographE3:** Section of rat ovary showing **theca (th)**,**Corpus luteum (CoL)** and**Growing follicle (Grf)**
Micrograph E4; Section of rat ovary showing **primordial follicle (Pf)**

Observations

Table 1; Summary of Properties of ovarian vertebrate tissue

SN	Properties				Fish	Toad	Lizard	Bird	Rat
1.	Capsule				+	+	+	+	+
2.	Germinal Epithelium				-	-	-	+	+
3.	Cortex								
4.		a.	Theca		+	+	+	+	+
5.	Medulla								
		b.	Lacunae		-	-	+	+	+
		c.	Follicle						
				Primordial	+	+	+	+	+
				Primaries	+	+	+	+	+
				Secondary	+	+	+	+	+
				Graafian	+	+	+	+	+
		d.	Corpus						
				Luteum	-	-	-	+	+
				Albicanthes	-	-	-	+	+
		.		Atreticum	-	-	-	+	+
		e	Granulosa layer						
				Single layer	+	+	+	-	-
				Multilayer	-	-	-	+	+

+Present; - Absent

tissue stroma with intervening blood vessels. Individual follicles are in varying stages of maturation, from primordial to graafian typestable 1 and micrographs **E1** and **E2**. The primordial follicles are lined by a single layer of fattened cells encasing each oocyte unlike the single layer of cuboidal epithelium seen in the primary follicle. The secondary follicle is enclosed by several layers of granulosa cells and a centrally located pale nucleus. The most mature follicle, the Graafian follicle presents a conspicuous antrum and corpus oophorus which wraps around the oocyte as shown in **E2**. The pellucidal membrane separates the ovum from the granulosa cells. Distinct blood vessels are present in the intervening ovarian stroma. A layer of peritoneum composed of flattened to cuboidal cells lines the outer aspect of the ovaries **E3** and **E4**.

Comments

This study, which compared ovarian morphology, revealed several significant findings especially the occurrence of multiple stages of germ cell development, which provided vivid anatomic histologic basis for the argument of a common ancestral origin of vertebrates.[6]

Figueiredo et al., 2008 had further demonstrated that while fish oogonia had a relatively large rounded to spherical nucleus, primary oocytes were characterised by the presence nucleoli as was observed in this study.[7] Similarly, the perivesicular stage, characterized by abundant perinuclear nucleolias revealed here, was not new as Handoni (1999); Cakici and Uncunu 2007 had previously noted similar findings.[8-9] Prominent yolk vesicles were a remarkable feature of a stage in the developing oocyte-yolk vesicle stage. These cytoplasmic vesicles which varied in sizes have been shown to contain lipid droplets and proteinous molecules which obscure the outline of cytosolic organelles with cellular maturity.

A peculiarity of fish ovary was the presence of one or two follicular cell layers. Both Rahemo/Al-Shatter and Haddani (1999) had commented that the outermost component of the two layered follicular layer represented the theca in fish while the inner layer was the granulosae.[9,10] A similar pattern of follicular layer was also documented in toad ovary characterised by an indistinct flattened granulosa and theca as had been recorded by Kanamadi and Saidpur in 1982; Sandapur and Nadkarni 1974.[11,12]

The variable ova sizes graduating from the earliest forms, oogonia to mature vitellogenic stages observed in the index study was not new. Though lefts (1974) and Guvaya (1979) had argued their existence of previtellogenic stages amphibians, Follet and Redshaw, 1974, clearly demonstrated the yolk sac stages in *Bufomelanostictus*.[13-15]These authors further noted that the content of the yolk nucleus was essentially similar to the micro molecules of reptilian, avian and mammalian oocytes. Also specified in this study was the occurrence of a single pleomorphic albeit, bland nucleus, whose histologic features were often obliterated by the cytoplasmic vacuoles especially in the yolk/ vitelline stage. Although multi-nucleolarity had been demonstrated by Brown and David, 1968; Al-Mukhtar and Webb 1971; and Dumont 1972, this characteristic was not obvious in this research. [16-18]The abundant intracytoplasmic vesicles are likely to have obscured observation of this prominent feature of amphibian oocytes.

Multi-vacoulation was also recorded in reptilian mature oocytes, although in this case, granulation was finer and less intensely eosinophilic. Machado-Santos et al. arrived at a similar conclusion in 2015 while documenting their observations in a species of South American Alligator in Brazil.[19] They further demonstrated that each oocyte, depending on its stage of development, was enclosed by a layer of flattened to cuboidal epitheliod cells (corresponding to granulosa cells) which were closely associated with a connective tissue stroma which separated the granulosa cells from the Zona pellucida covering the oocytes (Tumkiratiwong et al 2012, Guraya 1989, Calderon et al, 2004, lance 1989, Urbe and Gullette 2008).[20-24]Norris explained that these granulosa cells imbibed lipids (probably arising from progesterone and Estrogenic receptor) with development of the follicle and eventual transformation to Corpus luteum. Noris further demonstratedthat luteinized post ovulatory follicles eventually transformed to Corpora albicanthes, atreticum were not uncommon in reptiles thus sharing the existence of these structures as observed in birds and mammals.[25]

The ranges of follicles, from primordial to vitelline forms, were also evident corroborating the report of Vieira et al.[26] While the oogonia were observed in the cortical aspects of the ovary as earlier established by Calderon et al 2004, previtelline stages characteristically transversed the ovarian stroma, extending from the cortical aspects to the hilum/medullary zone characterised by thin walled blood vessels and one or more cystic spaces (Chordolacunar pattern).[27]This chordolacunar arrangement was not new asCallbaut1988; Ceriani and Wyneken 2008; had earlier opined that this vascular-cystic characteristic was not peculiar to reptiles but also occurred in higher vertebrates suggesting that the framework/design was consequence of a transformation into a more finely regulated and improved oocyte maturation.[28-29]

Besides possessing lacunae, reptilian ovaries also displayed some degree of ambivalence, sharing the propensity for multiple ovulations i.e. displaying the capacity for oviparity with lower vertebrate species (fish and amphibians) and birds, their immediate relatives in the evolutionary tree. Perhaps, the conclusions of Bull and Vogt,1981; Wibbels et al. 1991who confirmed that physical parameters such as temperature strongly influenced gonadal function in reptiles unlike in the avian class, evidently displayed a distinction, although in part, between lower and higher vertebrates whose ovarian activities were strongly influenced by genetic factors (Defalco and Capel, 2009).[2,30-31]

In his review, Johnson revealed that increased exposure to light at sexual maturation stimulated the release of gonadotropin releasing hormone (GnRH) whose activity was further indirectly modulated by ovarian steroid hormones. The fallout of this cooperation of hormones was development of multiple follicles (occurring as cluster cohorts) as was presented in the discussion of lower vertebrates (Rahemo and Al-Shatter,2012).[10]The consequence is that though single eggs were laid at any given time, eggswere produced in clusters especially in the breeding season. This characteristic of birds could be accounted for by the existence of mitogen activated protein kinase (MAPK).[32]

It is also noteworthy that the ovarian follicle displayed features similar to the more primitive vertebrates such as the existence of a single layer of granulosa cells unlike the more advanced multilayer of mammals.[33]Thecordo-lacunar arrangement was however ever more pronounced that for mammals. Callebaut 1988; Carianiand Wyneken, 2000 had earlier associated the persistence of existence of this structure with follicular maturation.[34,28] This explains the prominence of this component of avian ovarian tissue where there are abundant follicles many of which are in very similar phases of maturation. It is also important that scant atretic bodies were recorded in this study. Johnson and Woods (2009) postulated that mitogen-activated protein kinase (MAPK) signalling influenced maintenance of the developing follicle in an undifferentiated state.[32]

Follicle stimulating hormone (FSH) has been identified as an inducer of insulin-like growth factor binding protein protease (pregnancy associated plasma protein-A) which in the presence of estradiol, facilitates development of the dominant follicle with exclusion of others which eventually atrophy by apoptosis.[35] While the un-ovulated follicles of intermediate sizes form atretic follicles, the remains of the ovulated follicle is transformed into a luteal body. These forms were evident in the cortical aspect of the rat ovary in this study. Interestingly, for the first time in the series, growing and tertiary follicles displayed a multi-layered granulosa cell structure. Besides nutrition, other possible roles of the multi-layered granulosa cell patterns characteristic of mammals, is yet to be established. Also evident in mammals was the fascinating ontogenic progression from primordial to graafian and ultimately corpus albicanthes/ atreticum. The cycle was complete unlike in the earlier described vertebrates were maturation was arrested in the follicle (primordial to primary stages). A more advanced form of reproduction in which a single egg was ovulated such that all possible nutritive apparatus was directed at this follicle, occurred in mammals.

In conclusion, the index study has demonstrated several significant findings especially the occurrence of multiple stages of germ cell development in the female vertebrate gonads. The observation of multiple granulosa cell layers only in mammals confirms the advancement of this class over all the others. Adaptation to aquatic or terrestrial environments further provided explanations for existing differences. These observations provided a vivid histologic basis for the argument of a common ancestral origin of the animal phylum albeit in a background of adaptational influences.

Conflict of interests

The author wishes to declare that there is no conflict of interests in this publication.

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