



QUAIL MUSCLE SPINDLE

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ABSTRACT

The muscle spindles in birds, like duck, chicken, canary and pigeon were studied by authors briefly before. Science quail is a migratory bird and very little work was published on it's spindle, so this work was aimed to carry out the structure of the muscle spindle of *Coturnix coturnix* quail. Seven adult quails, *Coturnix coturnix*, were used in the present investigation. The results of the present study are based on the examination of spindles in a total of ten pronator superficialis muscles. Muscles were processed to obtain serial paraffin sections and were used for the detailed study of the general structure of their spindles, measurements and for the study of their ultrastructure. The present study showed no great variation in the number of muscle spindle in each muscles, the spindle length was proportional to the muscle length, two types of muscle spindles in quail, the single and the system types, one type of intrafusal muscle fibers. Mono and multifibrillar muscle spindles were observed but tandem spindles were not seen. Collagen fibers were observed around each intrafusal muscle fibers, however elastic fibers were not seen. One may conclude that quail muscle spindle have different structure than other avian muscle spindles.

Keywords: muscle spindle, Quail, intrafusal muscle fibers

INTRODUCTION

The term muscle spindle 'Muskelspindel' was first described as groups of relatively small intrafusal muscle fibers, enclosed within a fusiform connective tissue capsule [1]. The density of these receptors are usually related to the function of the muscle, so that those muscle controlling the fine movements have more muscle spindles than those used for controlling coarse movements[2]. In birds, [3] the spindle density per muscle varied widely in different muscles of the same bird, Coracotriceps muscle of the pigeon revealed a remarkable higher muscle spindle density than that of any muscle [4]. In chicken muscle spindles of were significantly higher in the anterior latissimus dorsi (ALD) muscle than in the posterior latissimus dorsi (PLD)[5].The same authors found that the muscle spindle had a uniform distribution through the ALD muscle and , uneven distribution in the PLD with the highest density near the region of entry of a nerve.

Three arbitrary regions (A, B and C) of muscle spindle was determined [6]: The (A) is equatorial and juxtaequatorial regions; the(B) region extends from the end of the periaxial space to the end of

the capsule; the(C) corresponds to the extracapsular region. Working on muscle spindle of the domestic chicken[5] and duck [3]showed that the outer capsule consisted of multilayered of 4-6 flattened cells with elongated nuclei and were lined by a basement membrane, they resembled and were linked with perineural cells of the nerve bundle that innervate the spindle. The cytoplasm of these cells had a lamellated cisternae of rough endoplasmic reticulum, microfilaments, pinocytotic vesicles and some ribosomes .The inner capsular cells at the mid equatorial region showed large ovoid nuclei , extensive branching of thin long processes that overlap forming numerous layers around each intrafusal fiber. There was no basement membrane lining the inner capsular cells and the processes. Elastic fibers were associated to cytoplasmic processes of the inner capsule in murine and human muscle spindles [7]. However, collagen fibers were associated with the capsule of the avian muscle spindle [7], particularly of chicken [5].

Intrafusal fibers were differentiated into nuclear bags and nuclear chains in

spindles of birds [8, 9, 10, 11]. One type of intrafusal muscle fibers could be identified in duck muscle spindles [12]. Tortoise muscle spindle has also only one type of intrafusal fiber Ragab [13]. About the number of intrafusal fibers within the spindle of birds, [5, 10, 12, 14] showed that the number of intrafusal muscle fibers per spindle varied. Monofibrillar muscle spindle was observed in the extensor digitorum communis and ALD of chicken, although, the extensor pollicis muscle and PLD did not contain a monofibrillar muscle spindle [5]. One paired muscle spindle complex was observed in the distal third of PLD muscle of chicken [5]. However, one (it contained two muscle spindles) tandem spindle was observed in the wing muscle of the domestic duck [12]. The comprehensive study of intrafusal muscle fibers in chickens, pigeons, and canaries did not mention finding any tandem spindles [12]. The muscle spindles in birds, like duck, chicken, canary and pigeon were studied by authors before. Science quail is a migratory bird and very little work was published on its spindle, particularly of quail, so it was decided to carry out the structure of the muscle spindle of *Coturnix coturnix* quail.

MATERIALS & METHODS

Seven adult quails, *Coturnix coturnix*, were used in the present investigation. The results of the present study are based on the examination of spindles in a total of ten pronator superficialis muscles. Six muscles, from three quails, were processed to obtain serial paraffin sections and were used for the detailed study of the general structure of their spindles but measurements were carried out on only three of these muscles. One muscle, from a different quail, was taken, prepared and stained to be examined for the elastic fibers in the spindles. Three more muscles from three different quails, were used for the study of their ultra structure, using a JEOL 100S transmission electron microscope.

The birds were killed with ether inhalation and the pronator superficialis muscles were exposed and separated from the surrounding structures. The muscles were fixed *in situ* by immersion in suza fluid [15] for 30 min, to prevent contraction and keep the original length of the muscles, after that they were carefully separated from their bony attachment and immersed in the same fixative for further 2.5 h. Dehydration was started by immersing the specimens in two changes, 30 min

each, of 95 % ethyl alcohol to which iodine crystals were added until the alcohol was dark brown in color to remove mercury deposits from the muscles. The dehydration was then completed using three changes of absolute alcohol over a period of 1 hand the proximal ends were then labeled. The specimens were cleared in 2 changes, 15 min each, of benzene and were finally infiltrated in 3 changes, 30 min each, with plasticized paraffin wax at 60°C for 1.5 h prior to embedding in the same, but fresh, plasticized paraffin wax. A rotary microtome was used to cut serial transverse sections 10µm in thickness. Each albuminized microscope-slide had ten consecutive sections arranged in one row, so that each slide represented 100 µm of the muscle length. Section cutting always started at the origin of the muscle. Consecutive slides were alternately stained with the following methods: **i- Hematoxylin and Eosin** stain was used for the study of the general structure of the spindles [16]. **ii- Curtis's Ponceau S** substitute for van Geison was used for the staining of collagen and muscle fibers [17]. **iii- Elastic fibers stain:** The orcein stain was used for the demonstration of elastic fibers in the spindles [18].

- For **electron microscopic examination**, Three quails were anaesthetized by ether inhalation, the pronator superficialis muscles were then exposed, cut and immediately fixed by immersion in 5% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 7.3, [19], and post fixed in 1% osmium tetroxide for one hour, then dehydrated in ascending grades of ethanol. After immersion in propylene oxide, the specimens were embedded in epoxy resin mixture. At first, 0.25-0.5 µm semithin sections were cut, picked up on glass slides stained with toluidine blue [13] and examined by light microscopy. Ultrathin sections (80-90nm thickness) were stained with uranyl acetate and lead citrate [20,21] and were examined and photographed with JEOL 1010 transmission electron microscope.

Measurements

-The length of the spindles and the capsules, in serial sections were obtained by counting the number of appropriate consecutive 10µm sections.
-The standard deviation of the mean (S.D.) was calculated according to the following formula: 2

$$S.D. = \sqrt{\frac{\sum (EX)^2}{n} - \frac{(\sum EX)^2}{n^2}}$$

Where \bar{X} = sum of values recorded
 n = number of values

The arithmetical mean \bar{X} is defined as

$$\bar{X} = \frac{\sum X}{N}$$

RESULTS

Particularly interesting was the result that the number of spindle capsules, hence the spindles, were closely equivalent in the three muscles (range 32 - 37 muscle spindle capsules per muscle). The spindles were located in the proximal and middle thirds of the muscles. All the spindles in the muscles were arranged, so that the intrafusal muscle fibers ran parallel to the extrafusal muscle fibers (Table 1). The mean spindle length for the 30

spindles present in the three muscles was 1.3mm (S.D.± 1.6) ranging from 840 μm to 1.8 mm (Table.2). The mean capsular length for 30 spindle capsules measured in the three muscles was 0.9 mm (S.D.±1.9) ranging from 620μm to1.3 mm. When each capsular length was expressed as the percentage of its spindle length, the mean value for the 30 capsules was 73.5%. (S.D ±12.1) ranging from 59.5% to 83.4% (table.2). This indicates that the capsule covered a proportionally large part of the spindle length and that the intrafusal fibers extended for a short distances beyond the capsule.

Table (1): Number and distribution of the muscle spindle capsules in each of the three muscles studied in serial transverse sections.

Muscle No.	Total no. of muscle spindle capsules	No.& percentage of spindle capsules in the proximal third		No.& percentage of spindle capsules in the middle third		No. & percentage of spindle capsules in the distal third	
		No.	%	No.	%	No.	%
M.(1)	37	No.	24	No.	13	No.	-
		%	64.8	%	35.3	%	-
M.(2)	32	No.	22	No.	10	No.	-
		%	68.7	%	31.2	%	-
M.(3)	34	No.	20	No.	13	No.	1
		%	58.8	%	38.2	%	3
Total	103	No.	66	No.	36	No.	1
		%	64	%	35	%	1

Table (2): Mean data of the length of 30 spindles studied in the serial sections of the three muscles.

Items	Spindle length	Length of capsule	% ofCapsule length Spindle length
Mean (S.D.±)	1.3mm (±1.6)	0.9mm (±1.9)	73.5% (±12.1)
Range	840μm-1.8mm	620μm-1.3mm	59.5%-83.4%

As far as the spindle capsules are concerned, two types of quail muscle spindles were recognized at the present

study. The common type was the single type which is composed of intrafusal muscle fiber or fibers

surrounded by a single outer capsule of the muscle spindle (fig.1:1) and the rare type was the system type which has more than one capsules enclosed in a common outer capsular sheath (fig.1:2& 1:4). The system type of muscle spindle found in the present study did not contain more than 2 muscle spindle capsules enclosed in a common capsular sheath (fig.1:3). In each of the three muscles examined, no more than three muscle spindles of the system type was observed. As far as the number of intrafusal muscle fibers in a spindle is concerned again two types of muscle spindles were recognized a monofibrillar type containing a single intrafusal muscle fiber in the single or the system type of muscle spindles (fig.1:3&1:4) and a multifibrillar muscle spindle containing multiple intrafusal muscle fibers (fig.1:1).

In the present study each spindle capsule was made up of two components, the outer capsular sheath and the inner capsular sheath (fig.1:1). The outer capsular sheath which enclosed the fluid filled space as well as all the intrafusal muscle fibers, was made of a thin connective tissue fibers containing collagen fibers and the flat capsular cells (fig.1:5). The cell of the outer capsular sheath had a flat nucleus

(fig.1:1). In between the capsular lamellae blood vessels (fig.1:2) were observed and nerve fibers were occasionally seen to penetrate the capsule (fig.1:5). In muscle spindle system the parallel muscle spindle capsules were enclosed in a common outer capsular sheath (fig.1:2). The adjacent capsules of a system lied side by side so that each capsule remained separate from the adjacent one (fig.1:2) or enclosed for a short part of their course together in a common capsule. As it was said before the outer capsular sheath of a muscle spindle deep in the muscle showed a connection with the perimysium (fig.1:6C). In the equatorial region of quail's muscle spindle, the fluid filled space was seen to be traversed by trabeculae to divide the fluid-filled space into incomplete compartments (fig.1:1&1:2). The fluid filled space as well as the trabeculae traversing it and the capsular sheath were gradually reduced at the juxtaequatorial and polar regions until they completely disappeared (fig.1:6E). In contrast, the inner capsular sheath was disposed so that each intrafusal muscle fiber had its own sheath consisting of multiple cell layers with flattened nuclei which

separated the individual intrafusal muscle fiber from each other (fig.1:1).Serially sectioned quail's spindles were carefully examined for elastic fibers in sections stained with orcein. It was observed that they did not contain elastic fibers in both the equatorial and the polar regions (fig. 2:1& 2:2). However, elastic fibers were stained in the walls of the blood vessels and were seen as discrete longitudinally arranged fibers parallel to the longitudinal axes of the vessels (fig. 2:1& 2:2). Therefore the absence of elastic fibers in relation to the intrafusal muscle fibers was definitely not due to failure of the stain.

Transverse sections from the equatorial region of muscle spindles were examined under the electron microscope. Two capsular sheaths were recognized according to their position. The outer capsular sheath which enclosed the fluid- filled space and all intrafusal muscle fibers and separated it from the surrounding extrafusal muscle fibers and the inner capsular sheath which closely enclosed each individual intrafusal muscle fiber and fine fluid-filled space (fig,3:1). The outer capsular sheath, which enclosed the fluid- filled space as well as all the intrafusal muscle fibers, was made of

concentric layers of very thin flattened cells with highly irregular cytoplasmic processes (fig.3:2). The cell of the outer capsular sheath had a flat, nucleus with occasional nucleolus and a very thin extensive sheet of cytoplasm (fig.3:2). A prominent feature of the outer capsular cells was the presence of basal lamina (fig.3:2). The layers of the outer capsular cells were separated by spaces containing collagen fibers running in different directions (fig.3:2 & 3:3). The inner capsular sheath consisted of multiple cell layers, each of which was made up of single cell closely enclosing the individual intrafusal muscle fiber and fine fluid- filled space(fig.3:3). The ultrastructural features of the cytoplasm of both outer and inner capsular cells were the presence of multiple pinocytotic vesicles and ribosomes (3:2& 3:3) but the only main ultrastructural difference between both capsules was the absence of the basal lamina in the inner capsular cell (fig.3:2 &3:3). Spindle system of quail showed 2 muscle spindle units enclosed in a common outer capsular sheath separating it from the surrounding extrafusal muscle fibers. The adjacent capsules of a system lied side by side so that each

spindle had its characteristic outer capsule. (fig.3:4). About the fluid filled space the present electron microscopic study showed that the fluid-filled space was traversed by trabeculae formed by cytoplasmic processes of the inner capsular cells

and this space was markedly increased in the equatorial region and decreased in the polar region (fig.3:4). Myelinated branches of nerve fibers were observed in the fluid filled space in the equatorial region (fig.3:1 & 3:4).

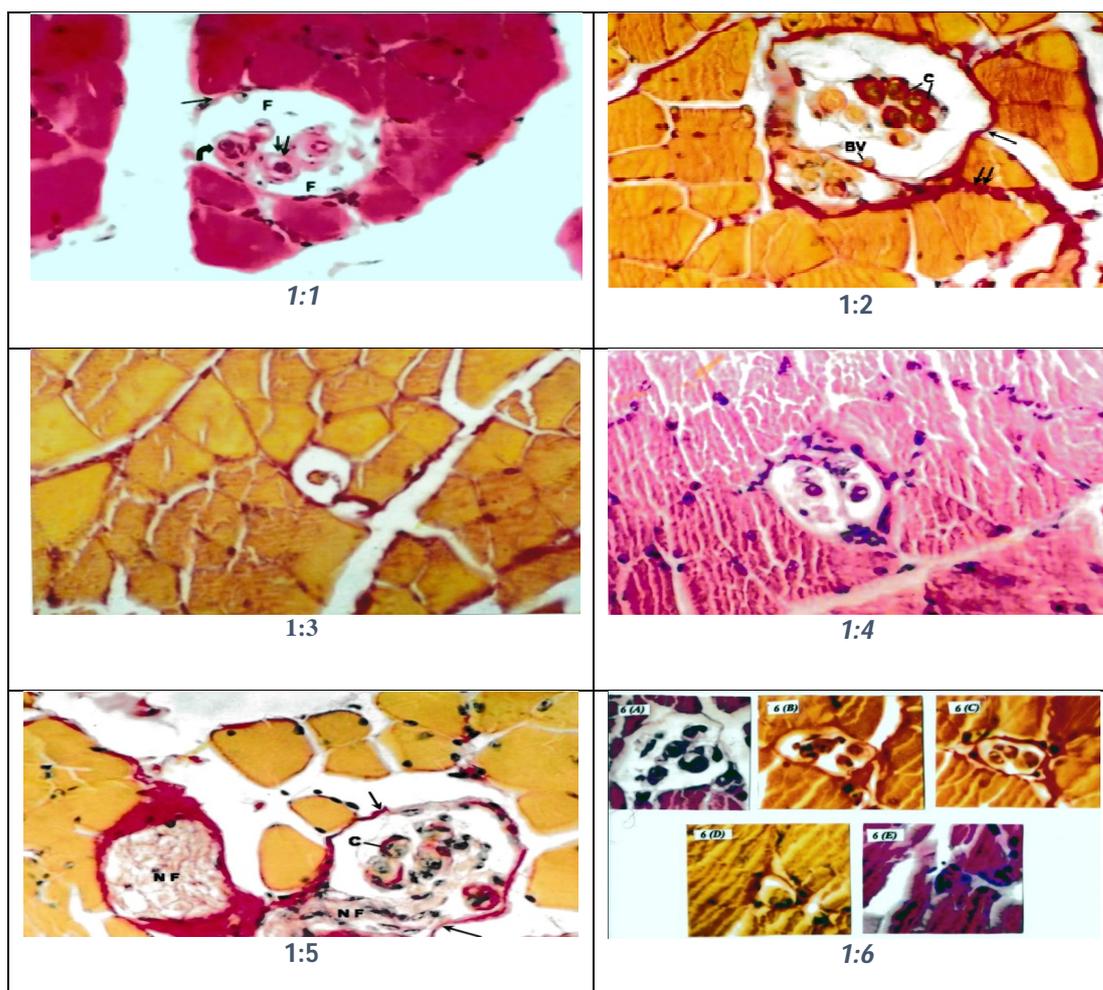


Fig.(1): A photomicrograph of a transverse section in the equatorial region of single type (1, 3,4) and system type (2,5) of muscle spindle showing , 1: 4 intrafusal muscle fibers, a fluid filled space (F), a thin outer capsular sheath (↓)containing cells with flatted nuclei, inner capsular sheath (↓↓) and the presence of three nuclei in one of the intrafusal muscle fibers (curved arrow)(HX.&E). 2: A common outer capsular sheath (→), the incomplete C shaped collagen rings, blood vessels (BV) traversing the capsular lamellae and the outer capsule continuous with the perimysium(↓↓)(Curtis s Ponceau S). 3 & 4:a monofibrillar muscle spindle (Curtis s Ponceau S.& HX.&E).5: A thin outer capsular sheath (→) formed of concentric connective tissue lamellae of collagen fibers alternated with the flat nuclei of the flat capsular cells, incomplete collagen ring (C), the nearby intramuscular nerve (NF) and the nerve fibers (NF) penetrating the outer capsule.6: Different parts of the same muscle spindle containing 4 intrafusal muscle fibers ,A, B,C& D in the capsular region and E in the extracapsular region. Notice; the gradual decrease in the fluid filled space and thickness of the outer capsular layer until they completely disappeared in E and that all intrafusal muscle fibers extend beyond the capsule and contain a peripherally situated single , flat and darkly stained nucleus in sections D&E. Section C shows a connection between the outer capsular sheath and the perimysium. (HX.&E & Curtis s Ponceau S.). (Mag. 1680X)

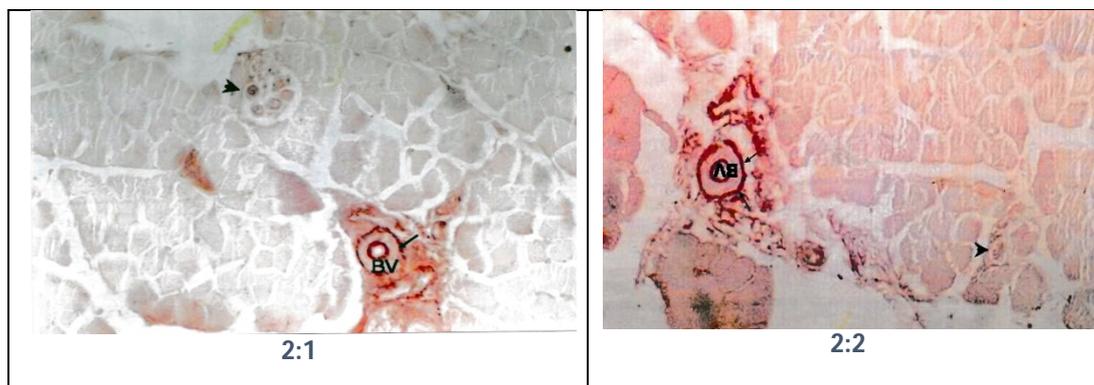


Fig.(2): A photomicrograph of a transverse section in the equatorial (1) and polar (2) regions of a single type muscle spindle (head arrow), showing; complete absence of elastic fibers in relation to the intrafusal muscle fibers, but present (→) in a nearby blood vessel(BV). (Orcein stain- Mag. 840)

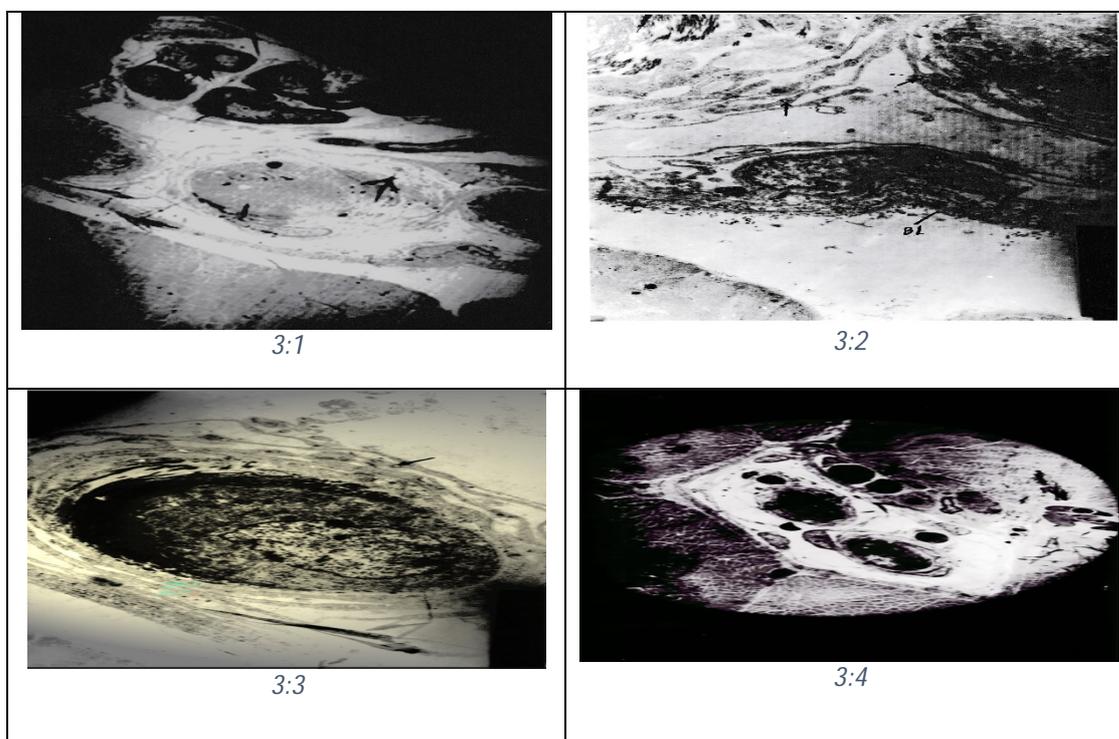


Fig. (4):- Representative electronmicrographic a transverse section of muscle spindles showing; 1: The outer capsular sheath that separates it from the extrafusal muscle fibers, the inner capsular sheath encloses individual intrafusal fibers and myelinated nerve fibers in the fluid filled space (Mag. 4.600X). 2: The outer capsular cell has a flat nucleus with a prominent nucleolus and extensive sheet of cytoplasm., the collagen fibers between layers of the outer capsular sheath, the presence of basal lamina (BL) in relation to the outer capsular cell and pinocytotic vesicles (↑) and ribosomes in the inner capsular cells (Mag. 11.500X). 3: Collagen fibers , multiple ribosome and pinocytotic vesicles(↑) within the inner capsule (Mag. 11.500X).4:A system type muscle spindle enclosed in a common outer capsular sheath, the inner capsular sheath enclosing the individual fiber, myelinated nerve fibers in the fluid filled space and collagen sheath around the intrafusal fibers in the equatorial region and the marked reduction in the fluid filled space and absence of collagen sheath around the intrafusal muscle fibers in the polar region (Mag. 11.500X).

DISCUSSION

The present study showed that in the pronator superficialis muscle taken from different quails contained 32 to 37 spindle per muscle. This means that there is no great variation in the number of muscle spindle in similar muscles. This result is in agreement with the results reported by [5] working on six pairs of ALD and PLD muscles of chickens. They found that the number of spindles ranged from 32 to 40 per muscle in the ALD and 16 to 20 in the PLD. Thus the density of muscle spindle was similar in each muscle, however, the ALD contained 52% more muscle spindles than the PLD and they explained that the large disparity in the density of muscle spindle in the two muscles may reflect their different functions. In the present study, the length of muscle spindles were measured and showed that the spindle length was proportional to the muscle spindle length. This result also reported by [22] who reported that the capsular length is proportional to the muscle spindle length.

Two types of muscle spindles in quail were recognized in present study, the single and the system types. This result is similar to the findings of other researchers working on spindles of

birds [5] and mammals [23, 24]. The present study did not show any tandem type of a muscle spindle which is similar to the result obtained by [10] on chickens, pigeons and canaries. However, [12] observed only one tandem spindles with two encapsulate regions in series out of 60 spindles and stated that tandem spindles are rare in avis. In the present study the quail muscle contained both monofibrillar and multifibrillar muscle spindles which is in agreement with the result of [5,12] who reported the occurrence of mono and multifibrillar muscle spindles in aves and that the number of intrafusal muscle fibers per spindle differ from muscle to muscle even in the same bird. Previous reports on both mammalian and avian muscle spindles revealed the presence of at least 3 morphologically different types of intrafusal muscle fibers, namely the nuclear chain and two types of nuclear bags, bag1 and bag2, fibers [27]. In avian muscle spindles, one to 4 types of intrafusal muscle fibers were reported to be present in different muscle spindles of different aves based on their variation in their diameter [5, 26,28,29,30.] Intrafusal muscle fibers in the present study could not be categorized into nuclear bag and

nuclear chain as the fiber showed no morphological differences corresponding to those distinguishing "nuclear bag" and "nuclear chain" muscle fibers in mammals. This result is similar to the result obtained by [10,12]. The present study showed that intrafusal muscle fibers of the quail muscle spindle at the equatorial region had two to three nuclei abreast for short distances which is similar to the work of the same authors. They explained these abreast of nuclei to be due to the slight overlapping in the arrangement of neighboring nuclei, as the nuclei of these fibers appeared as an irregular row in longitudinal sections and they were so close to one another that some slight overlapping occurred between them. The most important difference between avian and mammalian spindles was the relatively weak development of the nucleated region of the avian muscle spindle and as the differences in the viscoelastic properties of the equatorial and polar regions of an intrafusal muscle fibers of mammals were thought to give rise to the dynamic component of the primary afferent response to stretch thus they expected that the stretch response of avian spindle had a poorly

developed phasic component, and the stretch reflex as a consequence follows a somewhat different pattern from that in mammals and that physiological studies are needed to tell whether such differences between avian and mammals do exist [10]. All intrafusal muscle fibers in quail muscle spindles of the present study extended beyond the capsule. This result is similar to, fibers of the pigeon [26] and chicken [5]. This contrast to the observations by [10] for other avian muscle spindles who mentioned that intrafusal muscle fibers did not extend beyond the capsule.

The present study revealed that the spindle capsule of quail did not differ in its structure from those of all studied vertebrates in having multilayered outer capsular sheath common to all the intrafusal muscle fibers and inner capsular sheath for each individual intrafusal muscle fiber [5,7,25,26]. The present study demonstrated that quail muscle spindles did not contain elastic fibers, However, collagen fibers were observed. This is similar to what was reported in avian muscle spindles [5,8]. In the present study using light and electron microscopes all intrafusal fibers were partially encircled by a

collagenous fibers making a cap on a sensory ending in the equatorial region only of the muscle spindles that was formed of a tightly packed collagen fibers lied between the inner capsular cells and the sensory ending. This is in accordance with the finding of [5,26] who observed that cap in avian muscle spindle and suggested that this cap had a static protective function to prevent the terminal from displacement during movement and flying of birds.

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