

Age-Associated Changes in Dog Synovial Membrane Fibres, Capillaries and Nerves

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Abstract: Age related changes in the fibres, capillaries and nerves in the synovial membrane of the 24 (12 male and 12 female) Kangal hybrid-breed dogs were studied. The ages of the dogs varied and were classified in 3 groups; young (0-3 months), middle aged (3.5-6 months) and old (7 months to 6 years old). Synovial intimal layers were made of 1-2 layered intimal cells in the young group dogs and increased up from 3-5 to 2-6 multi-layered cells in the middle aged and the old dogs. The subintimas of young and middle aged groups were in areolar, adipose and areola-adipose types and were in fibrous type in the old group dogs. The collagen fibres of young group dogs were rare and scattered and they increased and occurred as bands and bundles in the synovium of the middle aged and old group dogs. The collagen fibres around the capillaries were rare in the young group and increased and occurred as collagen fibre bands and bundles around the capillaries and large blood vessels of middle aged and old group dogs. The thin elastic fibres increased from surface towards the deeper parts of the subintima of young dogs. In the middle aged group dogs, elastic fibres increased and thickened. The elastic fibres of the old group increased more and concentrated on the walls of the blood vessels and at the peripheries of the capillaries.

Key words: Dog synovial membrane, age-associated synovial changes, collagen fibres, elastic fibres, capillaries-blood vessels, nerves

INTRODUCTION

The synovial membrane is one of the fundamental parts of human and animal body articular cavities and plays a very critical role in joint movements in human and other animals. Different aspects of the synovial membrane have been studied extensively by Castor (1960), Fawcett (1986), Ghadially and Roy (1966), Krey *et al.* (1973), Levick and McDonald (1989), Rittig *et al.* (1992), Roy and Ghadially (1967), Sagirolu (1991a, b), Sagirolu *et al.* (1991), Shimizu *et al.* (1996), Shively and Van Sickle (1977), Watanabe *et al.* (1974) and Wyllie *et al.* (1964). The pathology of the membrane during rheumatic illnesses were investigated by Bleedorn *et al.* (2011), Chew *et al.* (1990), Hayashi *et al.* (2004), Henderson and Pettipher (1985), Knight and Levick (1984), Levick and McDonald (1989), Malone *et al.* (1991), Rittig *et al.* (1992), Roy *et al.* (1966), Sagirolu (1991b), Schumacher (1969), Thompson and Stockwell (1983), Watanabe *et al.* (1974) and Wright *et al.* (1989).

Synovial membrane shows variations in different mammalian animals (Bleedorn *et al.*, 2011; Cabrera *et al.*, 2008; Canpolat and Sagirolu, 2000; Castor, 1960; Comerford *et al.*, 2006; Fawcett, 1986; Hayashi *et al.*, 2004; Henderson and Pettipher, 1985; Krey *et al.*, 1973;

Levick and McDonald, 1989; Sagirolu, 1991a, b; Sagirolu *et al.*, 1991; Schumacher, 1969; Shimizu *et al.*, 1996; Shively and Van Sickle, 1977; Watanabe *et al.*, 1974; Wright *et al.*, 1989; Wyllie *et al.*, 1964; Wynne-Roberts and Anderson, 1978). Furthermore, many changes and abnormalities related with aging and several disease occur in morphology of synovial membrane (Bleedorn *et al.*, 2011; Canpolat and Sagirolu, 2000; Castor, 1960; Chew *et al.*, 1990; Fawcett, 1986; Henderson and Pettipher, 1985; Knight and Levick, 1984; Levick and McDonald, 1989; Malone *et al.*, 1991; Rittig *et al.*, 1992; Roy *et al.*, 1966; Sagirolu, 1991b; Sagirolu *et al.*, 1991; Schumacher, 1969; Shively and Van Sickle, 1977; Thompson and Stockwell, 1983; Watanabe *et al.*, 1974; Wright *et al.*, 1989; Wyllie *et al.*, 1964; Wynne-Roberts and Anderson, 1978). Now a days, studies on synovial membrane were concentrated on electron microscopic investigations of ultrastructure, changes caused by illnesses, ruptures and treatment (Bleedorn *et al.*, 2011; Cabrera *et al.*, 2008; Castor, 1960; Comerford *et al.*, 2006; Hayashi *et al.*, 2004; Rittig *et al.*, 1992; Roy *et al.*, 1966; Shimizu *et al.*, 1996; Wright *et al.*, 1989; Wynne-Roberts and Anderson, 1978). Therefore, studies on age associated changes in the synovial membrane have prime importance in understanding of

rheumatic and other age related illnesses in both human and animals (Bleedorn *et al.*, 2011; Canpolat and Sagirolu, 2000; Castor, 1960; Fawcett, 1986; Hayashi *et al.*, 2004; Henderson and Pettipher, 1985; Jilani and Ghadially, 1986; Rittig *et al.*, 1992; Sagirolu, 1991a, b; Sagirolu *et al.*, 1991; Shively and Van Sickle, 1977; Watanabe *et al.*, 1974; Wright *et al.*, 1989; Wyllie *et al.*, 1964; Wynne Roberts and Anderson, 1978).

This study is planned in two parts; investigation of age related changes in the synovial membrane cells and fibres, capillaries and nerves of dogs.

MATERIALS AND METHODS

Twenty four Kangal hybrid dogs, 12 male and 12 female were used in this experimental work. They were grouped in three:

- Young animals (5 dogs) were 0-3 months old and weighed 2700-3500 g
- Middle aged animals (6 dogs) were 3.5-6 months old and their weights were between 7-14 kg
- Old and very old animals (12 dogs); 7 months to 6 years old, weighed between 10-33 kg

Dogs were classified with the aid of veterinarian and according to classifying methods used in the earlier researches (Canpolat and Sagirolu, 2000; Jilani and Ghadially, 1986; Sagirolu *et al.*, 1991; Thompson and Stockwell, 1983).

The dogs were killed by intracardiac injection of Nembutal and then left and right knee joints of the dogs were incised. Suprapatellar tendons, quadriceps and other muscle connections and joint capsule were incised. The flaps containing the patella were reflected and synovial membranes over the medial and lateral of the infrapatellar pad and parapatellar region were collected speedily (Canpolat and Sagirolu, 2000; Castor, 1960; Chew *et al.*, 1990; Ghadially and Roy, 1966; Jilani and Ghadially, 1986; Knight and Levick, 1984; Krey *et al.*, 1973; Levick and McDonald, 1989; Roy *et al.*, 1966; Roy and Ghadially, 1967; Sagirolu *et al.*, 1991; Schumacher, 1969; Thompson and Stockwell, 1983; Watanabe *et al.*, 1974; Wyllie *et al.*, 1964).

Tissues were placed on filter paper as intimal layers positioned on top (Canpolat and Sagirolu, 2000; Ghadially and Roy, 1966; Jilani and Ghadially, 1986; Krey *et al.*, 1971, 1973; Levick and McDonald, 1989; Roy *et al.*, 1966; Roy and Ghadially, 1967; Sagirolu *et al.*, 1991).

For microscopy, tissues were fixed in 10% formalin and Helly's solution, dehydrated in increasing

concentrations of ethanol, cleared in xylol and embedded in paraffin (Jilani and Ghadially, 1986; Luna, 1968; Roy *et al.*, 1966). About 5-6 μ m thickness synovial membrane sections were prepared and stain used Haematoxylin-Eosin, Crossman's triple stain, Resorsin-Fuchsin and Kluver-Barrera. Stained sections were studied using BH 2-Olympus photomicroscope (Bradbury and Gordon, 1977; Crossman, 1937; Disbrey and Rack, 1970; Luna, 1968; McManus and Mowry, 1960).

RESULTS AND DISCUSSION

In the young dogs (1st group), 0-3 months old: Macroscopically, synovial membrane appeared white, smooth, thin and glistening. Intimal layer of the articular cavity is formed 1-2 layers of intimal cells (Fig. 1). The cells were platy or oval shaped fibroblast-like and macrophage-like cells (Fig. 1). Subsynovial (subintimal) layers of this group of dogs showed changes from the surface towards depths, areolar, adipose, fibrous and

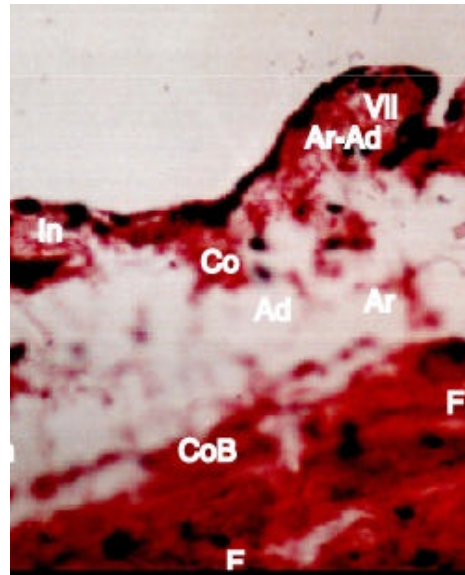


Fig. 1: Synovium of young (0-3 months old) dogs. Synovial membrane Intima (In) towards articular cavity is made of 1-2 layered cells. Intimal cell layer extends towards the articular cavity as Areola-Adipose (Ar-Ad) connective tissue bearing Villus (Vil). In the surfical layer of subintima, Collagen fibres (Co) are scattered or clustered. In the deeper layers Areolar (Ar) and Adipose (Ad) connective tissues are present. In the deepest layers, Collagen fibers form Bundles (CoB) and Fibroblasts (F) are present. Haematoxylin-Eosin, X40

dominantly areola-adipose types (Fig. 1 and 2). Collagen fibres were observed as scattered small clusters in superficial subintima and as bundles in deeper parts (Fig. 1 and 2). The amount of elastic fibres gradually increased towards deeper layers of surficial subintima and there were abundant connective tissue cells among the fibres (Fig. 2).

There were plenty of capillaries and small arterioles among and just under intimal cells. In deeper subintima, thicker blood vessels with varying radii were present (Fig. 2).

Second group (middle aged animals = 3, 5-6 months old):

The collagen fibres were as either scattered or as bundles on the surficial subintima. In deeper subintima, the collagen fibres were as bundles and bands. The collagen fibres were concentrated around blood vessels (Fig. 3 and 4). Elastic fibres were found accumulated in internal elastic membrane of an artery (Fig. 4).

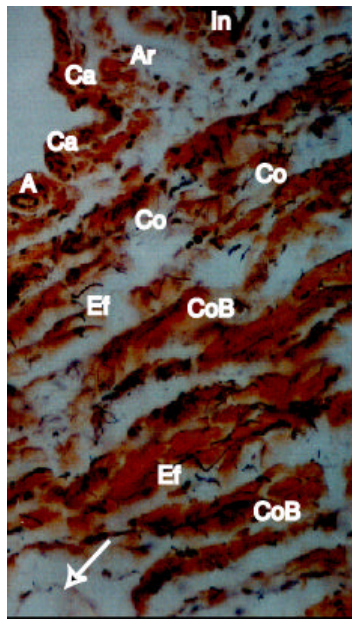


Fig. 2: Various shaped and sized villus is present in first group dogs. 1-3 or 2-5 layered Intimal cells (In) occur. Many Capillaries (Ca) and small Arteriole (A) are present among the cells and close to intima. A large villus filled with Aerolar connective tissue (Ar) is observed. Subintima layers include plenty of scattered Collagen fibres bundle (Co) and towards deeper layer Collagen fibres (CoB) increase. From surficial layer towards deeper layer of subintima Elastic fibres (Ef) gradually increase and there is a large blood vessel (arrow) between connective tissue cells. Resorsin-Fuchsin, X20

From intima towards superficial and deep subintima, various capillary, arteriole, vein and vein-like structures increased was observed (Fig. 3 and 4). In addition vascularity in subintima was more crowded in comparison to subintima of first (young) group (Fig. 5 and 6).

In this group (middle aged), peripheral nerves and among them capillaries were emplaced in deeper parts of synovial membrane (Fig. 6).

Third group (old group = 7 months to 6 years old animals):

Macroscopic appearance of the synovial membranes of this group were pastel greyish coloured and had rough surface. Although, platy cells were present in places cell shapes were dominantly cubic and polygonal and the intimal layer was formed by 2-6 layers of cells (Fig. 7). The subintima of old animals group was areolar and more fibrous connective tissue character (Fig. 7-9).

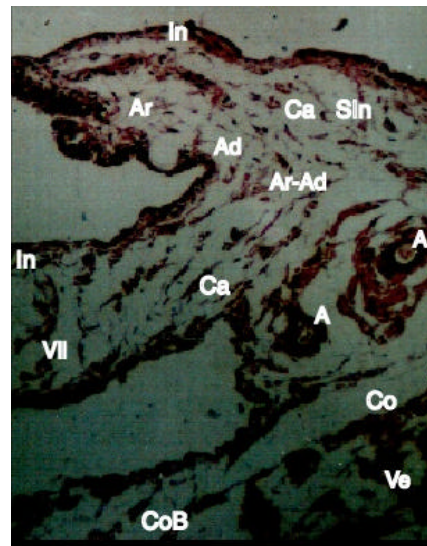


Fig. 3: Microscopic view of synovial membrane of middle aged (2) group. Synovial membrane Intimal layer (In) bears 1-3 or 3-5 layered intimal cells. Various shaped and sized Villus (Vil) included either Areolar (Ar), Adipose (Ad) or Areola Adipose (Ar-Ad) connective tissues. In subintima, scattered Collagen fibres (Co) and in deeper subintima layers Collagen fibres Bundles (Co and CoB) were present. The connective tissues were generally Areola-Adipose (Ar-Ad) type and the bundles are present in Subintima (SIn). From intima towards subintima and deeper subintima, various Capillaries (Ca), Arterioles (A) and Venules (Ve) were present with varying diameters. Collagen fibre Bundles (CoB) surrounding large arterioles were observed. Haematoxylin-Eosin, X10

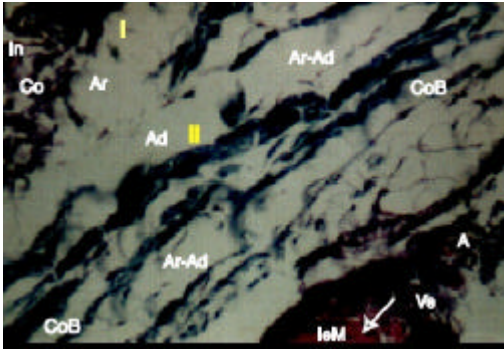


Fig. 4: Occurrence of second group (middle aged) dog's synovium. Synovial Intima (In) was formed of 1-2 layered cells. Collagen fibres (Co) just under intima were rather concentrated. Collagen fibre Bundles (CoB) were seen in the Areolar connective tissues (Ar) of deeper layers (I and II) and in the Areola Adipose (Ar-Ad) and Adipose (Ad) of subintima. An artery (arrow) in the deep subintima had Internal elastic Membrane (IeM) and surrounding it a Venule (Ve) and Arterioles (A) were present. Collagen fibre Bundles (CoB) and various connective tissues were present as surrounding blood vessels. Crossman's triple, X20

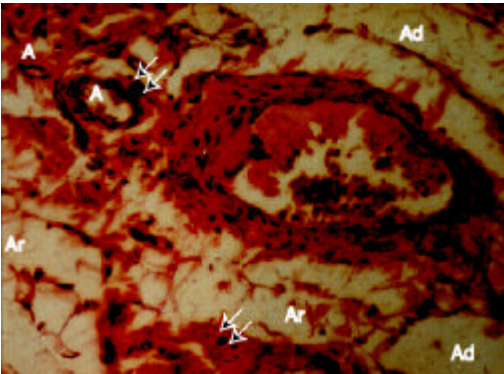


Fig. 5: The appearance of subintima of middle aged group dogs. Subintima was lined with Areolar (Ar) and Adipose (Ad) connective tissues. In comparison with young group dogs, vascularity increased in the deeper parts of subintima. A large, probably a mast, cell (pointed with double arrow) was present close to Arteriole (A) wall. Haematoxylin-Eosin, X20

At the surface the collagen fibres were scattered among intimal cells (Fig. 7) and the fibres graded into collagen bundles and band towards deeper parts (Fig. 7 and 8).

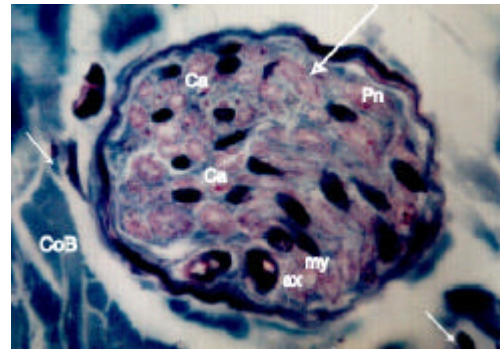


Fig. 6: Microscopic view of deeper layer of synovial membrane of second (middle aged) group. Note the peripheral nerve (long arrow) surrounded by Perineurium (Pn). Capillaries (Ca) were present among nerve fibres. Collagen fibre Bundles (CoB) around and blood vessels (arrow) among nerve tissues were present. Ax: Axon, my: myelin sheath. Crossman's triple, X100

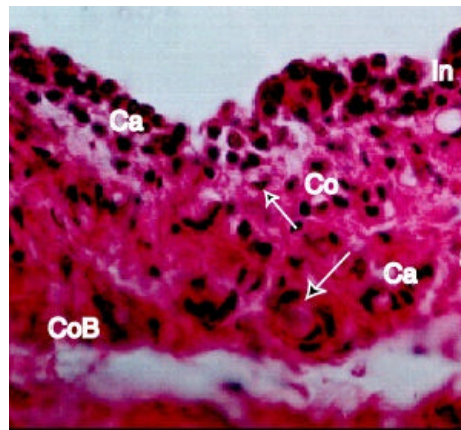


Fig. 7: Microscopic view of synovium of the old (7 month to 6 years old) dogs. The Intimal cell layer (In) was formed of 3-6 layered. Among the multi-layered intimal cells Capillaries (Ca) were present. The subintima was changed to fibrous connective tissue. In subintimal layer, Capillaries (Ca) among various connective tissue cells and blood vessels (arrow) were present. Collagen fibre (Co) and Collagen fibre Bundles (CoB) were concentrated in the vicinity of blood vessels. Haematoxylin- Eosin, X40

Elastic fibres in synovial membranes of old animal group were increased both in general and on the vascular walls, in comparison with the young group (Fig. 8).

The capillaries among intimal cells of the old group were decreased in comparison with those of young group

(Fig. 7). However, from subintima towards deeper parts numbers and radii of capillaries and other vascular structures increased (Fig. 7 and 8).

The peripheral nerves were observed deepest part of the synovial membrane and among collagen fibres bundles and close to the capsule of the knee joint (Fig. 9).

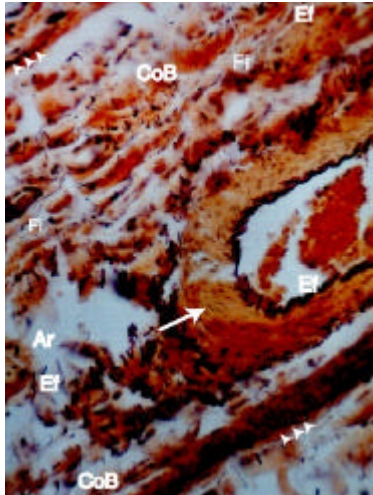


Fig. 8: Macroscopic appearance of deeper parts of synovium of old group dogs. Subintima changed to Areolar (Ar) and Fibrous (Fi) connective tissues. Collagen fibres occur as thick Bundles (CoB) and bands (arrow heads). Elastic fibres (Ef) increased and thickened in both general structure of synovial membrane and in the walls of blood vessels. Connective tissue cells and collagen and elastic fibres were present close to a blood vessels (arrow). Resorsin-Fuchsin, X20

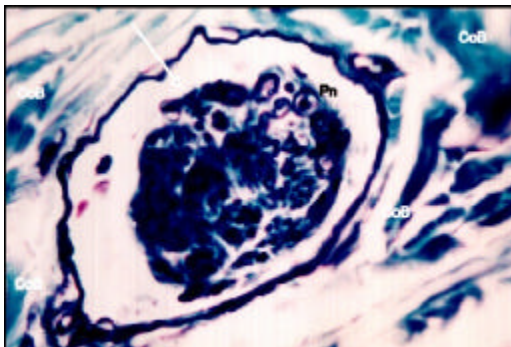


Fig. 9: Peripheral nerve (long arrow) close the capsule of synovial membrane of old group dogs. Collagen fibre Bundles (CoB) placed around nerve and Perineurium (Pn). The connective tissues were in fibrous connective tissue texture and connective tissue cells decreased. Kluver-Barrera, X40

The studies on age related changes in human and experimental animal synovial membranes have not been able to distinguish remarkable degenerations (Castor, 1960; Rittig *et al.*, 1992; Roy *et al.*, 1966; Wright *et al.*, 1989; Wynne-Roberts and Anderson, 1978). However, the most of studies on animal and human synovial membranes were on young subjects (Bleedorn *et al.*, 2011; Cabrera *et al.*, 2008; Castor, 1960; Comerford *et al.*, 2006; Fawcett, 1986; Ghadially and Roy, 1966; Hayashi *et al.*, 2004; Henderson and Pettipher, 1985; Krey *et al.*, 1971, 1973; Levick and McDonald, 1989; Roy and Ghadially, 1967; Schumacher, 1969; Shimizu *et al.*, 1996; Shively and Van Sickle, 1977; Thompson and Stockwell, 1983; Watanabe *et al.*, 1974; Wright *et al.*, 1989; Wyllie *et al.*, 1964; Wynne-Roberts and Anderson, 1978).

This study investigates age related changes in dog synovial membrane with light microscopic methods. Recent research on dog synovial membrane are generally on the changes related disease, ruptures and medical treatments (Bleedorn *et al.*, 2011; Cabrera *et al.*, 2008; Canpolat and Sagiroglu, 2000; Castor, 1960; Comerford *et al.*, 2006; Hayashi *et al.*, 2004; Henderson and Pettipher, 1985). Therefore, this study will contribute in understanding age associated changes in the synovial membrane of dog. Findings of this study are outlined and correlated with the findings of earlier resaerch on the subject.

Macroscopic appearance: The synovial membranes of the young group dogs were pearly white coloured, smooth, glistening and thin (Jilani and Ghadially, 1986; Krey *et al.*, 1971; Sagiroglu *et al.*, 1991; Wynne-Roberts and Anderson, 1978). In older dogs the colour of the synovium changed to greyish, rough and pastel. Jilani and Ghadially (1986), Sagiroglu (1991a, b) and Sagiroglu *et al.* (1991) described similar macroscopic appearances for experimental animals and human.

Intima: The synovial intimal layers generally were made of 1-2 layered intimal cells in young dogs, 1-2 layered or 3-5 layered in middle aged group and 2-6 layered in the old group.

Subintima: From the surface towards depths of the subintima of young and middle aged dogs were mostly as areolar, adipose and areola-adipose connective tissue types. In the synovial membranes of the old group subintima areolar were mostly changed to fibrous connective tissues.

It is noted that subintima type could change depending on the functions of and pressures and impacts on the synovium (Fawcett, 1986; Henderson and Pettipher, 1985).

Collagen fibres: In the studied dog synovial membranes, the collagen fibres were rare and scattered in the young group and the fibres increased gradually and made bundles and bands in middle age and old groups. Collagen fibres were plenty among intimal cells of the old group. The collagen fibres around capillaries were rare in the young group dogs and plenty and as bundles and bands in the old group.

Collagen fibres in human are very similar to of dogs and according to Castor (1960) they do not show changes with aging. In experimental animals, the aging causes in an increase of collagen fibres (Canpolat and Sagiroglu, 2000; Ghadially and Roy, 1966; Sagiroglu *et al.*, 1991; Watanabe *et al.*, 1974).

Elastic fibres: In the young group of the studied dogs, thin elastic fibres increased from surficial subintima towards deeper layers. The elastic fibres further increased and thickened, in the middle and old aged group dogs synovial membrane. In the old group, elastic fibres increased in general structure of synovial membrane and on the walls of the blood vessels and peripheral of the capillaries.

Elastic fibres are very rare in human synovial membrane and increases with age (Castor, 1960; Jilani and Ghadially, 1986; Wynne-Roberts and Anderson, 1978). Earlier research (Canpolat and Sagiroglu, 2000; Castor, 1960; Sagiroglu *et al.*, 1991; Thompson and Stockwell, 1983) stated that elastic fibres were present in the synovial membrane of rabbits and dogs and according to Sagiroglu (1991b) and Sagiroglu *et al.* (1991) elastic fibres in rabbits synovial membrane decreased with aging.

Capillaries: In the young group of studied dogs, capillaries were present abundantly among intimal cells and layers just under the cells. The capillaries were either absent or rare among the synovial intimal cells of old dogs. However, in a trend from young to middle then to old, capillaries and larger vessels increased and had larger radii towards the deeper parts of subintima. From young to old dogs, vascularity gradually increased in deeper subintima. The vascularity was as capillaries among the intima and as large vessels in the peripheries of old dogs. Similar structures and age related changes were described in human synovial membrane by Fawcett (1986), Krey *et al.* (1971), Wynne-Roberts and Anderson (1978).

In experimental animals, capillaries were more abundant in young animals and exhibited different trends with aging of the animals (Canpolat and Sagiroglu, 2000; Knight and Levick, 1984; Krey *et al.*, 1973; Sagiroglu *et al.*, 1991; Schumacher, 1969; Shively and Van Sickle, 1977).

Nerves: Nerve fibres with or without myelin of the dog synovial membrane were detected close to the joint capsule in all the animals of the studied three groups. Those peripheral nerves were observed close to the blood vessels and collagen fibres of the deeper layers of subintima.

In the textbooks, it is stated that the nerves in the synovial membrane conveyed to the blood vessels (Fawcett, 1986; Shimizu *et al.*, 1996).

Although, the existences of nerve tissues in synovium were known (Shimizu *et al.*, 1996), the changes due to aging and variations in different species were not described (Henderson and Pettipher, 1985).

CONCLUSION

The capillaries and large blood vessels increased gradually from the young group towards the middle aged and the old group at the deeper synovium. The nerve fibres in the synovial membranes of the studied 3 group dogs were quite close to the joint capsules. These peripheral nerves placed very close to the blood vessels and collagen fibres of subintima.

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