

# The microarchitecture of the tissues of the shoulder joint in dogs

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Up to the present time practically not studied the peculiarities of formation of cartilage in the various compartments of the shoulder joint in dogs, as well as zones of least resistance joints in General. Available studies mainly use visual diagnostic methods, without applying morphological and morphometric. There is no information about age-related cytomorphological transformations occurring in the joint in normal and pathological conditions. In this regard, the aim of our study was to determine the zone of risk of damage to the shoulder joint in dogs. Studies were conducted on dogs and cadaver material, selected from giant, large and medium-sized breeds. Arthroscopic studies of dogs were performed, as well as light microscopy of histological sections of freshly treated samples of joint tissues. As a result of trauma to the joint is transformed physiological synovial folds in sklerozirovanie a pathological form that occurs in German shepherd dogs over 5 years of age in 60% of cases. The process of mineralization of the matrix and the appearance of multiple foci of ossification occurs asynchronously in the cartilage of the head of the humerus and the articular cavity of the scapula. Osteoplastic processes in the area of articulation occur unevenly. Cartilage coating of the articular surface of the shoulder blade is characterized by a more uniform distribution than the articular rotation of the humerus head. The zones of risk of damage to the structures of the shoulder joint in dogs were determined. These include: cranial and medial compartments of the joint, the caudal area of the cartilaginous coating of the humerus, the caudal area of the articular surface of the scapula cavity. The data obtained make up for the information about the diagnosis and risk factors in the development of shoulder joint pathologies in dogs of large and giant breeds. They should be used in clinical diagnosis and surgery of the musculoskeletal system in animals.

**Keywords:** shoulder joint, dog, tissue, cartilage, capsule, bone, arthroscopy, microscopy, shoulder bone, shoulder blade.

## Introduction

The study of morphological equivalents and pathological transformation of skeletal system and tendons and ligamentous apparatus apparatus is one of the topical issues in clinical morphology and veterinary medicine. Nowadays the importance of its solution is becoming more and more urgent because of the spread of scapulohumeral periarthritis among large and giant dog breeds that causes limping of unknown etymology. The most common pathology is osteochondritis dissecans (Rudd et al., 1990; Shea et al., 2013; Slater et al., 1991; Uozumi et al., 2009). A great number of diagnostic studies have been done, but, in general, only clinical approach is used thus not considering the structure-functional features of a particular dog that have impact on its pathogeny and pathomorphism.

The structure of coverage of cartilaginous joint is known (Pavlova et al., 1988; Omeliyanenko et al., 1991, 1995) though the features of cartilage formation in different parts of joint and structure of capsule are still not explored nowadays.

A large number of studies are devoted to arthropathy of an elbow joint (Hefti et al., 1999; Nagura et al., 1969; Shea et al., 2013; Uozumi et al., 2009; McCoy et al., 2013; Ytrehus, Ekman et al., 2004; Ytrehus, Haga et al., 2004). However, the importance of the shoulder joint cannot be overestimated as it is the joint that provides mobility on a chest extremity. The lameness connected with pathologies of this joint will lead to the dysfunction of all extremity.

Thus, the present study is aimed to define the area of the least tissue stability in dogs' glenohumeral joint

coverage taking into consideration the features of postnatal ontogenesis.

The following research questions are central to the present study:

What are the morphological characteristics of scapulohumeral articulation in postnatal ontogenesis of dogs?

Where are the areas of the least tissue stability in the glenohumeral joint coverage located?

How are the joint components interconnected in the case of joint pathology?

## Materials and methods

### Materials

The objects of this research study were dogs and cadaver material from giant breeds (Great Dane, Central Asian Shepherd Dog, Caucasian Shepherd Dog) large breeds (Russian wolfhound (Borzoi), Hortaya borzaya, Doberman) medium breed (German Shepherd, Half-breed German Shepherd) and also wolves from hunting farms. Clinical, arthroscopic and histological examination (microscopic and morphometric) of these breeds have been done. Overall the sample included 49 specimens aged 0–15 (the average age was 38 months).

### Procedure

The following were used to study the features of microarchitecture of glenohumeral joint tissue:

microdissection with binocular loupe and subsequent description and functional analysis of studied structures, arthroscopy, light microscopy of histological sections and statistical analysis of digital data.

### Arthroscopy study

For arthroscopy study KARL STORZ instrument was used. The joint was filled up with isotonic solution of sodium chloride and the intraoperative joint irrigation was done continuously. The arthroscope was 2,4 mm in diameter with angulation 30°.

The arthroscopy evaluation was done by cells: cranial, mesial and caudal with anatomical structures (tendons, grooves, ligaments) and condition of joint fluid (Petitt et al., 2008; Seimering et al., 1986; Van

Bree et al., 1998; Van Gestel et al., 1985; Van Ryssen et al., 1993).

Different forceps, curettes and hook clips were used as additional instruments for arthroscopy operation and diagnostic studies.

At arthroscopy Zoletil 100 (10 mg/kg) and “Xyla” (3 mg/kg) were used for sedative effect of animals.

### Histological examination

The samples of head of humerus joint cartilage and glenoid cavity of scapula, joint capsule, conjugate ligaments and muscles were studied with the help of light microscopy. Selected materials were fixed in 10% neutral formalin solution, its fixing properties are capable to form methylene bridges between polypeptide chains of tissue.

After that the samples were flushed with water-pipe for 24 hours, dehydrated in alcohol of various strength (60°–100°) and embedded in paraffin. After the studied fragments with bone tissue were fixed in paraffin they were decalcified in 5–7% nitric acid solution.

The serial paraffin sections (7–10 µm width) were made by universal automatic microtone HM-360 “Mikrom” (German) (Avtandilov, 1990; Semchenko, 2006).

The histologic section were hematoxylin and eosin stained by practical standard (Pavlova et al., 1988, Avtandilov, 1990) and then studied with light microscopy (Nikon).

Micromorphometry of structure was done using Nikon Eclipse E200 microscope with the help of certified program “Image Scope S”.

## Results and discussion

Histologically the capsule consists of 2 layers. The outer layer is fibrotic and the inner layer is sinovial (Figure 1).

It is shown that the joint capsule is of uneven thickness and becomes thin in the cranial part, it consists of 2 layers, which is the common of structure among studied dogs.

The structure of collagen-elastic layer of synovial membrane normally contains blood and lymph capillaries with narrow lumina (Figure 2). Collagen

Figure 1  
*Microscope slide of a shoulder joint capsule*

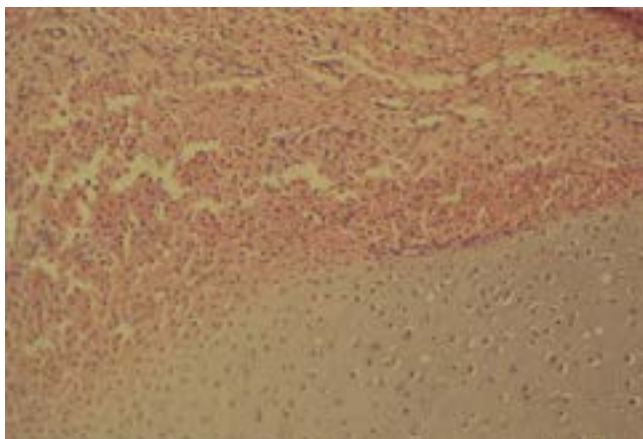


Figure 3  
*Pathological proliferation of a synovial villi due to joint traumatization. Arthroscopy*



Figure 2  
*Microscope slide of a synovial membrane of a shoulder joint. A large number of capillaries is visible*



Figure 4  
*Histotopography of the humeral bone head of a newborn puppy*



fibers and fibroblasts are in mural space. Such microarchitecture is determined by the fact that synovial membrane is instrumental in the trophic provision of articular cartilage, besides it removes metabolism products of chondrocytes.

The oedema and hyperemia of synovial membrane were among 25% of German Shepherd and 20% of Caucasian Shepherd Dog over 1 year of age; while the occurrence among these breeds over 6 years of age was 65% and 40% respectively.

The lump of pathologic forms of synovial folds as well as the local thickness of synovial membrane in the places of maximal rubbing were detected among animals with excessive thinning of cartilaginous coverage (Figure 3).

As a result of joint trauma physiological synovial folds transform into sclerosal pathological forms (in the cases of tendon incarceration of biceps, tenosynovitis and osteochondritis dissecans). This pathology was found among 60% of German Shepherds over 5 years of age, which may serve as a adaptation-compensatory mechanism for keeping stability of a pin-joint.

One of the main characteristics of a cartilage is the change of thickness according to its condition (Pavlova et al., 1988; Hall, 2005). Another meaningful factor of metabolic activity process in cartilage is the condition of chondrocyte nucleus. These cells actively synthesizing collagen and proteoglycans have big circular or oval shaped nucleus with even edges. The cells with pyknosis or rhexis of a nucleus, which indicate the cell necrosis, lose their regular circular shape while their edges become uneven. The thickness of cartilage was measured by dropping a perpendicular from subchondral bone plate to articular surface.

The articular cartilage of the newborn animals did not have zonal differentiation because of the absence of tested mechanical loading, although even at this age there are numerous ossification centres (Figures 4–12). The surface layer of cartilaginous tissue is full of chondrocyte without strict ordering (Figure 6). The

Figure 5

*Microscope slide of the humeral bone head of a newborn puppy. The multiple centers of an ossification are visible*

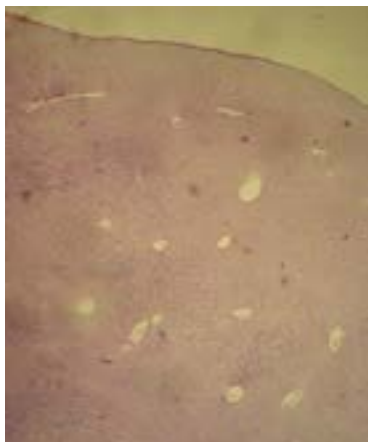


Figure 8

*Microscope slide of the humeral bone head of a newborn puppy. The ossification center is visible*

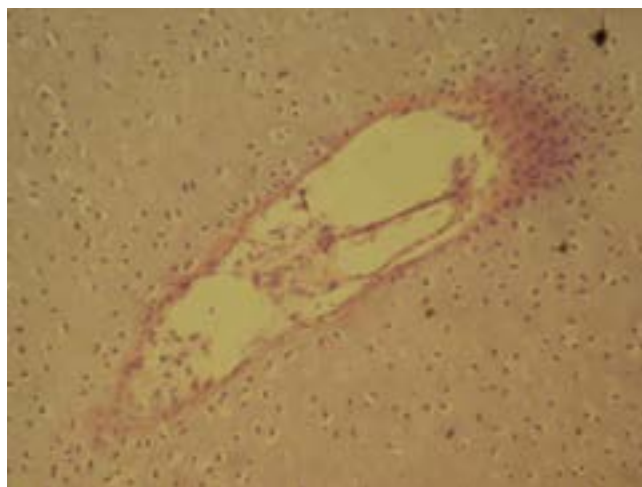


Figure 6

*Microscope slide of a humeral bone head of a newborn puppy. Surface layer*

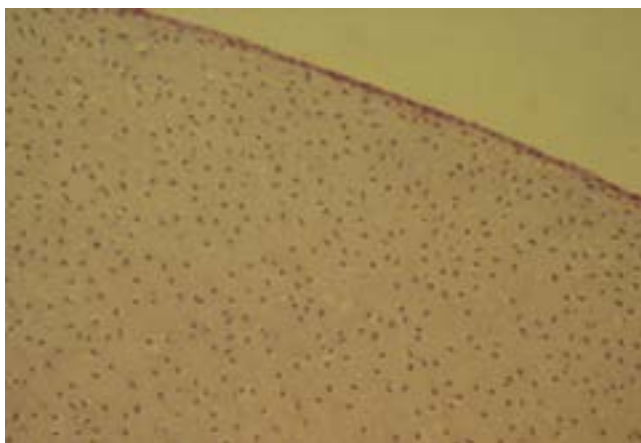


Figure 9

*Microscope slide of the humeral bone head of a newborn puppy. Formation of bone beams*

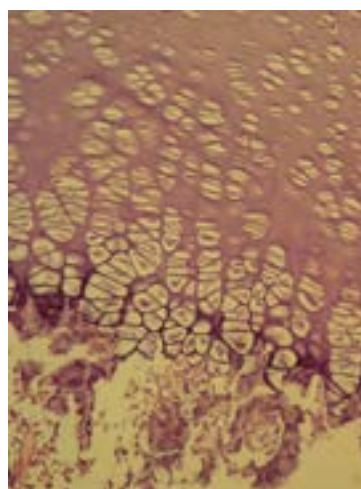


Figure 7

*Microscope slide of a humeral bone head of a newborn puppy. Deep layer*

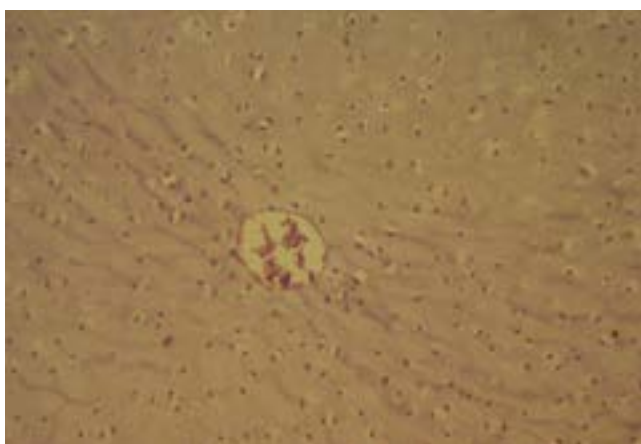


Figure 10

*Histotopography of the shovel of a newborn puppy*



Figure 11  
*Microscope slide of the articulate surface of the shovel (a newborn puppy)*

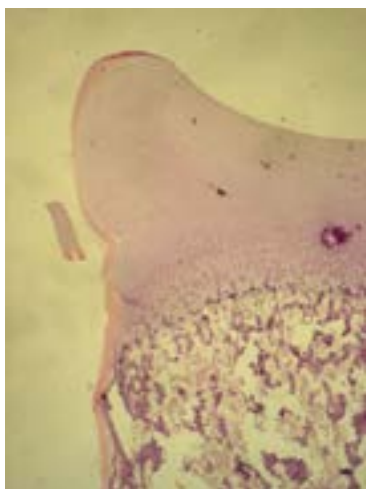
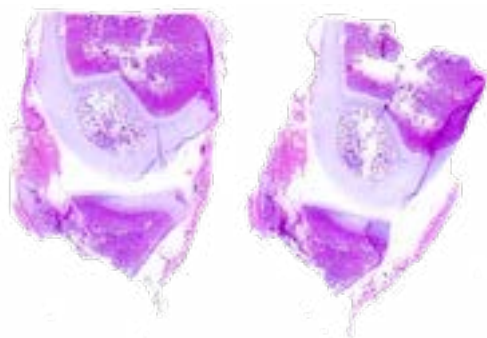


Figure 12  
*Microscope slide of the articulate surface of the shovel (a newborn puppy). Surface layer.*



Figure 13  
*Histotopography of the central and caudal parts of the shoulder joint (a puppy of 1 month)*



chondril balls were not found, although there were small groups of 2-3 cells (Figure 12). In deep layers the chondrocytes were hypertrophied with pyknotic nucleus (Figure 9). The fact that draws much attention is process of matrix mineralization and the appearance of numerous ossification centres. These processes are asynchronous in head of humerus cartilage and glenoid cavity of scapula: the differentiation of scapula articular cartilage outruns the differentiation of humerus (Figure 13). Moreover, the osteoplastic processes are not even. The trabecula of bone formation is strengthened in the central part of scapula articular surface (Figure 11), that reflects various intensity of metabolism in the same tissue cells having different location.

The cranial part of the humeral head cartilaginous covering is distinguished by the latest terms of structural formation. There are many (more than 10) centres of ossification in it (Figures 16–17). The central part occupies an intermediate position in the rate of formation between the cranial and caudal parts (Figures 18–19). It revealed an insignificant (1–2 in the field of view) number of ossification centres. In the caudal part, ossification centres are absent (Figure 22). The structural organization of the cartilage in the caudal part of the humeral head is approaching the definitive one. At the same time, the tidemark does not acquire pronounced tinctorial properties (Figures 20–21).

A slight thinning of the cartilaginous coating in the caudal zone of the humeral head is characteristic of all canines. However, in the case of the German Shepherd its thinning was detected starting from four months of age (Table). The hyaline cartilage of the

Figure 14  
*Cartilage of the head of a shoulder (central part). The ossification center is visible*

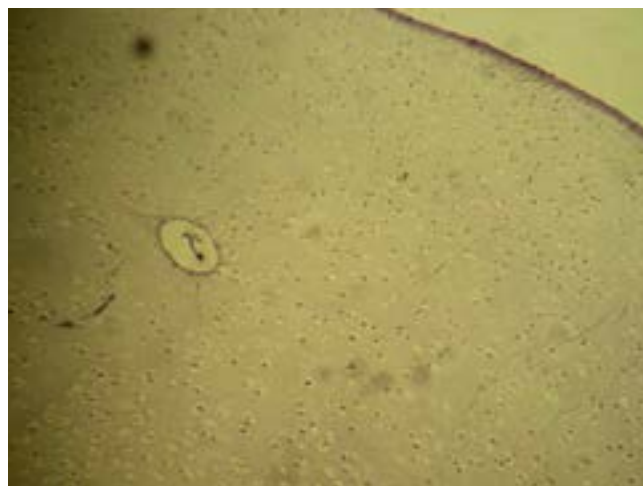


Figure 15  
*Formation of bone beams*

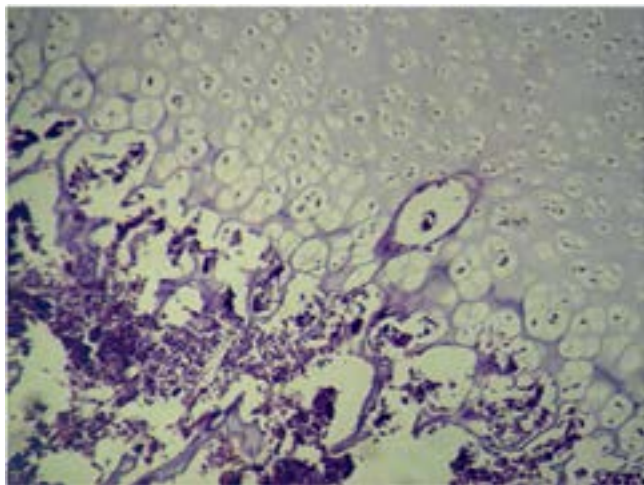


Figure 18  
*Histotopography of the central part of a humeral bone head (a four-months German shepherd)*

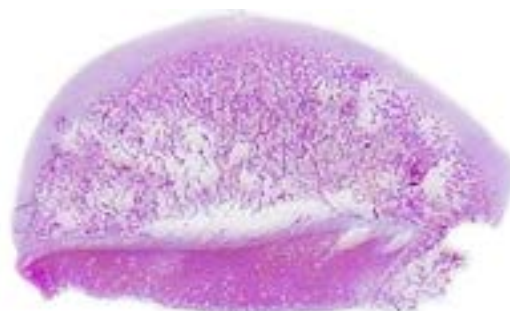


Figure 16  
*Histotopography of cranial part of a humeral bone head (a four-months German shepherd)*

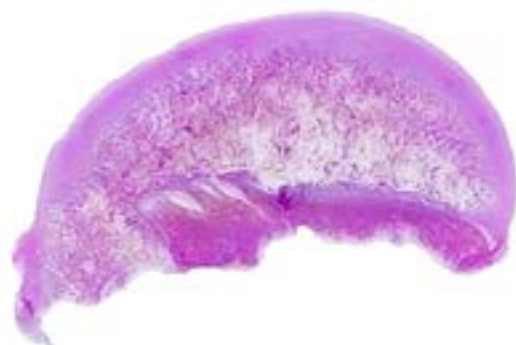


Figure 19  
*"Bubbly" chondrocytes of the central part of the humeral bone head*

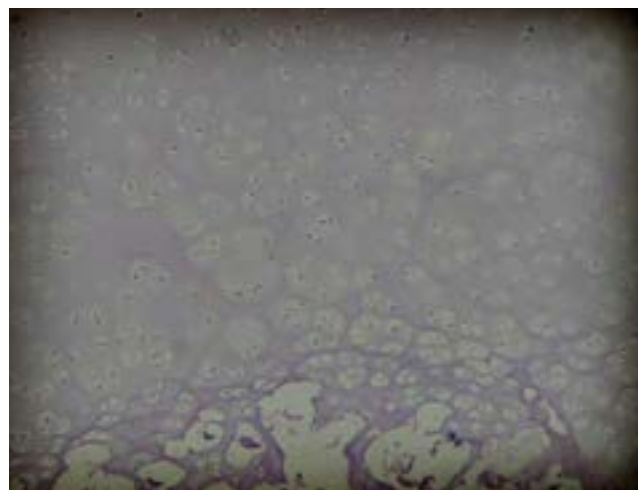


Figure 17  
*Deep layer of a cartilaginous covering of a cranial humeral bone head (a four-months German shepherd)*

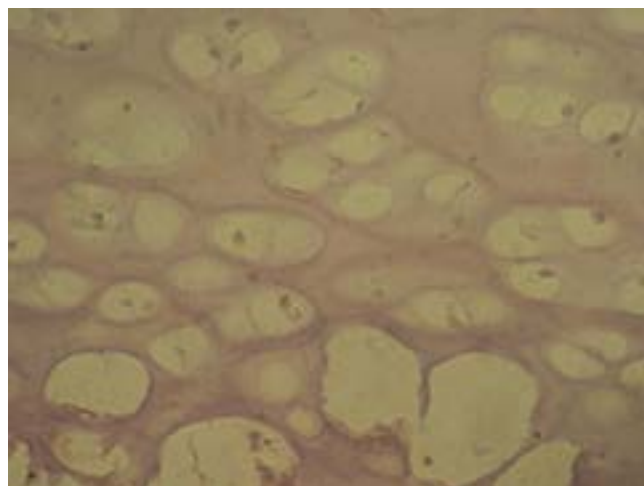


Figure 20  
*Histotopography of the caudal part of a humeral bone head (a four-months German shepherd)*



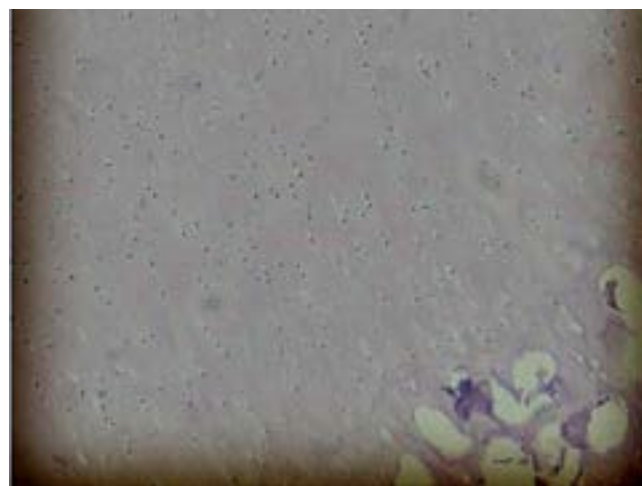
caudal area in the German Shepherd is thinner than in the central area by 22.5% while in the caudal area by 51.4%. In dogs of other breeds, the thickness of the cartilage covering in different compartments of the joint varies within 15%. The cartilaginous covering of the articular surface of the scapula is characterized by a more even distribution. Differences between the compared values are significant ( $P \leq 0.05$ ).

On the basis of the conducted research, the most vulnerable areas of the cartilage coating were identified, these include the caudal areas of the articular surface in the root of the scapula and the head of the humerus. That is why dissecting osteochondritis of the humeral head is diagnosed only in the caudal region of the joint.

When studying the morphogenesis of the shoulder joint, we have identified the zones of the least resistance in its anatomical components. These are the cranial and medial compartments of the joint, the caudal area of the humerus cartilage covering, the caudal area of the cartilage covering of the scapula articular surface, the cartilage-humeral ligaments, the tendon of the

Figure 21

*The caudal part is most closer to a definitive state. The division into three zones (superficial, medium and deep) is visible. Tidemark is formed*



the cartilage covering of the scapula articular surface, the cartilage-humeral ligaments, the tendon of the subscapularis muscle.

Table

*Micromorphological parameters of articular cartilage in dogs*

Age	Articular cartilage of the scapula, mkn			Articular cartilage of the humerus, mkn.		
	Cranial.	Centre	Caud.	Cranial.	Centre	Caud.
Newborn	1316,00 ± 0,53	1280,00 ± 0,55	1267,00 ± 0,55	1724,00 ± 0,41	1710,00 ± 0,41	1681,00 ± 0,
1 month	1298,00 ± 0,83	1237,00 ± 0,87	1184,00 ± 0,97	1706,00 ± 0,66	1654,00 ± 0,68	1440,00 ± 0,70
4 month	1256,00 ± 0,64	1179,00 ± 0,68	1048,00 ± 0,76	1684,00 ± 0,47	1534,00 ± 0,52	1397,00 ± 0,57
4 months (German Shepherd)	1124,00 ± 0,62	1063,00 ± 0,66	968,00 ± 0,72	1820,00 ± 0,55	1140,00 ± 0,59	884,00 ± 1,13
Older than 1 year	1023,00 ± 0,78	985,00 ± 0,81	921,00 ± 0,87	1259,00 ± 0,63	1138,00 ± 0,70	984,00 ± 0,81
Older than 6 years	344,00 ± 2,32	592,00 ± 1,35	809,00 ± 9,89	736,00 ± 1,09	625,00 ± 1,23	534,00 ± 1,49

subscapularis muscle. Since the cartilage-humeral ligaments and the tendon of the subscapularis muscle grow together with the capsule, their damage leads to the thinning or damage of the capsule.

The research findings:

1. The areas of the shoulder joint under the risk of the structure damage have been identified. These cranial and medial compartments of the joint, the caudal area of the humerus cartilage covering, the caudal area of

2. The difference in the postnatal ontogenesis of the humeral head cartilage is clearly observed from the age of four months.

## Conclusion

This study adds to the previous research on the occurrence of dissecting osteochondritis at the level of cartilage morphology and the characteristics of its formation in wolves, large and giant dog breeds.

Besides it addresses the gap in the knowledge on genetic predisposition of arthropathy (Coopman et al., 2008; Davidson et al., 2008; Emily et al., 2016; Ohlerth et al., 2016). It is obvious that considerable reduction of thickness of a cartilage at the age of 4 months leads to serious pathologies of a joint that manifest themselves at a young age (by 1.5–2). The present research confirmed the genetic determination of cutting osteochondritis of a humeral bone head; a set of studies are dedicated to the etiology of the latter.

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## Микроархитектоника тканей плечевого сустава у собак

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До настоящего времени практически не изучены особенности формирования хряща в различных отсеках плечевого сустава у собак, а также зоны наименьшей устойчивости сочленения в целом. Имеющиеся исследования в основном используют визуальные методы диагностики, не применяя морфологических и морфометрических. Отсутствуют сведения о возрастных цитоморфологических преобразованиях, происходящих в суставе в норме и при патологии. В связи с этим целью нашего исследования явилось определить зоны риска возникновения повреждения плечевого сустава у собак. Исследования проводились на собаках и кадаверном материале, отобранном от гигантских, крупных и средних пород. Были произведены артроскопические исследования собак, а также световая микроскопия гистологических срезов свежееизвлеченных образцов тканей сустава. Вследствие травматизации сустава происходит трансформация физиологических синовиальных складок в склерозированные патологические формы, что встречается у немецких овчарок старше 5 лет в 60% случаев. Процесс минерализации матрикса и появление множественных очагов окостенения происходит асинхронно в хряще головки плечевой кости и суставной впадины лопатки. Остеопластические процессы по площади сочленения протекают неравномерно. Хрящевое покрытие суставной поверхности лопатки характеризуется более равномерным распределением, нежели суставная поверхность головки плечевой кости. Определены зоны риска возникновения повреждения структур плечевого сустава у собак. К ним относятся: краниальный и медиальный отсеки сустава, каудальная область хрящевого покрытия плечевой кости, каудальная область суставной поверхности впадины лопатки. Полученные данные восполняют сведения о диагностике и факторах риска в развитии патологий плечевого сустава у собак крупных и гигантских пород. Их целесообразно использовать при клинической диагностике и хирургии опорно-двигательного аппарата у животных.

**Ключевые слова:** плечевой сустав, собака, ткань, хрящ, капсула, кость, артроскопия, микроскопия, плечевая кость, лопатка

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