

## PHYSIOCHEMICAL AND CYTOLOGICAL CHARACTERISTICS OF SYNOVIAL FLUID IN THE CARPAL JOINT OF MIXED-BREED GOATS

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### ABSTRACT

The carpal joint in goats is subject to diseases such as caprine arthritis encephalitis, leading to pain and a reduction in movement. Due to the scarce records of normal parameters, the aim of the present study was to assess the physiochemical and cytological make up of the synovial fluid in the carpal joint of 44 mixed-breed goat without joint disease. The following physiochemical characteristics were assessed: volume ( $0.94\text{mL} \pm 0.26\text{ mL}$ ), color (colorless to yellow), turbidity (46.50% were limpid), mucin

precipitation (firm coagulant in limpid solution) and concentration of total proteins ( $1.95 \pm 0.87\text{ mg/dL}$ ). Cytological assessment consisted of global and differential leukocyte counts. The mean global count was  $48.02 \pm 24.47\text{ cells}/\mu\text{L}$ . The differential count was  $25.07 \pm 15.86\text{ lymphocytes}/\mu\text{L}$  and  $16.31 \pm 12.63\text{ large mononucleated cells}/\mu\text{L}$ . It is suggested that the parameter values obtained in the present study may be considered normal for synovial fluid in the carpal joint of adult mixed-breed goats.

KEY WORDS: Carpus, goat, joint, synovia.

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### RESUMO

#### CARACTERÍSTICAS FÍSICO-QUÍMICAS E CITOLÓGICAS DO LÍQUIDO SINOVIAL DA ARTICULAÇÃO DO CARPO DE CAPRINOS MISTIÇOS

A articulação do carpo dos caprinos é alvo de enfermidades, como a artrite-encefalite caprina, levando à dor e à redução dos movimentos. Devido à falta de registro de parâmetros normais, este estudo avaliou a constituição físico-química e citológica do líquido sinovial da articulação do carpo de 44 caprinos sem raça definida, que não apresentavam doença articular. Os parâmetros físico-químicos avaliados foram volume ( $0,94\text{ mL} \pm 0,26\text{ mL}$ ), cor (incolor a amarelo), turbidez (46,50% apresentaram-se lípidas), precipitado de mucina (coágulo firme em solução límpida) e con-

centração de proteínas ( $1,95 \pm 0,87\text{ mg/dL}$ ). Os dados citológicos corresponderam às contagens global e diferencial de leucócitos. A contagem global média encontrada foi de  $48,02 \pm 24,47\text{ células}/\mu\text{L}$  e contagem diferencial com  $25,07 \pm 15,86\text{ linfócitos}/\mu\text{L}$  e  $16,31 \pm 12,63\text{ grandes células mononucleadas}/\mu\text{L}$ . Sugere-se que os valores obtidos nesta pesquisa sejam considerados parâmetros normais para o líquido sinovial do carpo de caprinos adultos sem raça definida.

PALAVRAS-CHAVES: Articulação, caprino, carpo, sinóvia.

## INTRODUCTION

Synovial fluid is a dialysate of plasma that also contains substances secreted from the joint tissue itself, including relatively high amounts of hyaluronic acid (PARRY, 1999). The main function of synovial fluid is to lubricate and nourish the joint cartilage, which has no blood vessels, lymph vessels or nerves (PARRY, 1999). Analysis of synovial fluid is performed in order to identify cytological and physiochemical abnormalities in suppurative and non-suppurative inflammatory conditions, hemorrhages, malignant tumors or infectious diseases. The abnormalities are used together with clinical and historical signs to determine the treatment and prognosis. Evaluations in series assist in the assessment of the response to therapy. The removal of accumulated fluid and inflammatory products from the distended joint capsule may relieve symptoms (KIEHL, 1997).

One of the advantages of a synovial fluid exam is the ease of execution, as it can be performed with a minimum of equipment in a short space of time (PARRY, 1999). WOODARD et al. (1982) reported that the main clinical abnormality in caprine arthritis encephalitis (CAE) in specimens of various ages (including six weeks of age) was the occurrence of hygroma. While this lesion is not specific to CAE, the frequency of hygromas was greater and the lesions more severe than that expected in goat populations unaffected by this disease.

As in other organic fluids, the assessment of synovial fluid may be divided into three categories, according to BOON (1997): physical (color, volume, turbidity), chemical (concentration of total proteins and formation of mucin precipitate) and cytological (nucleated cell count and swab analysis). A number of studies report the analysis of synovial fluid in dogs (BOON, 1997; PARRY, 1999), humans (SUGIUCHI et al., 2005; BRANNAN & JERRARD, 2006) and horses (VAN PELT, 1974) in either normal or pathological conditions. Laboratory findings determined a large number of mononucleated cells in the synovial fluid. Therefore, analysis of this fluid can assist in the diagnosis in distinguishing arthritis caused by CAE from other inflammatory joint diseases caused by bacteria or mycoplasmas, in which polymorphonuclear leukocytes are the predominant cells (WOODARD et al., 1982).

Synovial fluid in goats has been analyzed in the presence of arthritis encephalitis (WOODARD et al., 1982). However, the scarcity of studies on normal values of synovial fluid components in the carpal joint of goats hampers the interpretation of results.

Thus, the aim of the present study was to contribute toward knowledge on normal physiochemical and cytological characteristics of synovial fluid of the carpus in adult goats.

## MATERIALS AND METHODS

Forty-four male mixed-breed goats were used. The animals had the following characteristics: age between six and 18 months; no history of joint disease; obtained from the Buíque Municipal Slaughterhouse, state of Pernambuco, Brazil; with negative serological evaluation for lentivirus as determined by the agar-gel immunodiffusion test (Biovetech®).

Synovial fluid collection was performed on the carpal joint, from the right and left antimeres, in the antebrachiocarpal and mediocarpal portions of the joint, using a 25x8 hypodermic needle and 5-mL disposable syringe. Immediately following collection, the samples were homogenized, stored in a recipient with thermal isolation and promptly sent to the laboratory for analysis.

The synovial fluid from each joint was aspirated until completely drained for the evaluation of volume, color and turbidity. The concentration of total proteins was determined by refractometry, following the procedure described by MAHAFFEY (1992), after the addition of a 10% EDTA solution.

Analysis of the quality of the mucin precipitate was determined by the addition of 0.1 mL of 7N glacial acetic acid to 4 mL of distilled water in a test tube. The resulting solution (2.5%) was gently added to a 0.5-mL aliquot of synovial fluid, with care taken so that the sample did not come into contact with the wall of the test tube. The interpretation of the result was performed based on the criterion recommended by VAN PELT (1974): normal = firm coagulant in limpid solution; regular = soft coagulant in slightly turbid, yellowish solution; poor = small, friable mass in turbid, light yellow fluid; and very poor = few flakes in turbid, light to dark yellow fluid.

For the cytological analysis, EDTA was added to the synovial fluid samples. Global nucleated cell counts

were performed with a Neubauer hemocytometer (BOON, 1987). Swabs of synovial fluid were stained with Rapid Panoptic and analyzed under an optical microscope Olympus BX50<sup>®</sup>. Nucleated cells were classified as neutrophils, eosinophils, lymphocytes and large mononucleated cells.

All variables, except the categorical variables (color, turbidity, quality of mucin precipitate) and percentages obtained in the differential leukocyte count were analyzed for normality and homogeneity of variance and transformed, when necessary, prior to the statistical analysis. Regarding the variables investigated to determine possible significant differences between the right and left antimeres, multivariate analysis of variance (MANOVA) was used for volume, concentration of proteins and global count of nucleated cells. Univariate analysis of variance (ANOVA) was then applied in order to explain any differences detected by MANOVA. The remaining variables were compared using the chi-square test. Volume, concentration of total proteins, global count of nucleated cells and percentage values of the differential count of nucleated cells

(leukocytes) were submitted to the “bootstrap” re-sampling method with 1000 replications in order to establish the most reliable parameters, with a 95% confidence interval.

## RESULTS AND DISCUSSION

The samples analyzed were viscous and had a mean volume of  $0.94 \pm 0.26$  mL (Table 1). VAN PELT (1974) reports that the total volume of synovial fluid aspirated from any joint varies in proportion to joint size and its communication with another joint. SUGIUCHI et al. (2005) and BRANNAN & JERRARD (2006) state that viscosity is reduced in inflammatory states due to the reduction in hyaluronic acid polymers by lysosomal enzymes produced primarily by neutrophils as well as cells from the capsule and synovial cartilage. Variation in volume and composition of synovial fluid indicates a pathological alteration, especially inflammatory processes. In studies carried out on dogs, sample volumes ranged from 0.01 to 1 mL and from 0.20 to 1 mL for each joint.

**TABLE 1.** Physiochemical characteristics of synovial fluid in the carpal joint of mixed-breed goats

Characteristics	Mean	CI (95%)		SD	Minimum value	Maximum value
		LL	UL			
Volume (mL)	0.94	0.85	1.05	0.26	0.40	1.50
Total protein (g/dL)	1.95	1.57	2.30	0.87	0.80	3.60
Characteristics	Proportion		Minimum and maximum limits established			
Color	41.9% colorless		Colorless to yellow			
Turbidity	46.5% limpid		Limpid to semi-turbid			
Mucin precipitate	100% normal		Normal (firm in limpid solution)			

CI = confidence interval; LL = lower limit; UL = upper limit, SD = standard deviation

The color of the synovial fluid samples in the present study ranged from colorless to dark yellow: 41.90% colorless, 30.20% dark yellow and 27.00% light yellow. Regarding turbidity, 46.50% of the samples were classified as limpid, 41.90% were semi-turbid and 11.60% were turbid (Table 1). According to BRANNAN & JERRARD (2006), turbidity is generally related to the leukocyte count, with higher counts correlated to greater turbidity. Normal synovial fluid is limpid and its coloration ranges from colorless to

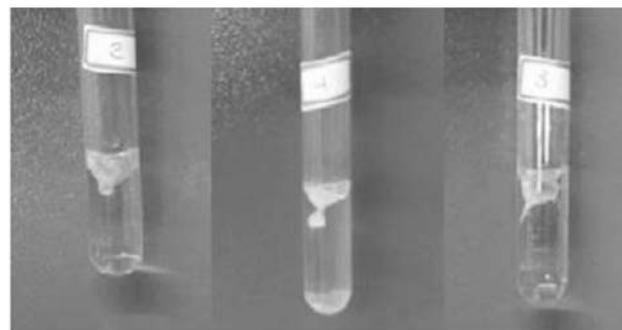
light yellow (BOON, 1997). According to WILKINS (1993), an increase in turbidity suggests an increase in white blood cell count and a change in color indicates either a hemorrhage or iatrogenic contamination by blood at the time of collection.

Mean protein value in the present study was  $1.95 \pm 0.87$  g/dL (Table 1). The concentration of proteins in synovial fluid is generally low and few studies report these values. However, a study carried out on dogs by FERNANDEZ et al. (apud PARRY, 1999) reports a

normal protein concentration between 1.80 and 4.80 g/dL. According to BANKS (1992), proteins with low molecular weight, which are immunologically and electrophoretically identical to plasma proteins, are found in low concentrations in synovial fluid. KOLB (1984) states that the amount of proteins in synovial fluid is approximately 1% and made up mainly of albumin, globulins and mucin. Higher protein values occur during inflammatory processes, along with the presence of larger proteins in the synovial fluid, such as fibrinogen.

The quality of the mucin precipitate in all samples analyzed was considered normal, based on the VAN PELT (1974) classification– firm coagulant in a limpid solution (Figure 1; Table 1). The mucin precipitate test indicates both quality and concentration of hyaluronic acid (BOON, 1997) and it is a reliable indicator of the polymerization of hyaluronic acid in synovial fluid and a qualitative measure of its concen-

tration in normal and pathological synovial fluid (VAN PELT, 1974). According to SUGIUCHI et al. (2005), in the presence of inflammation, enzymes in the synovial fluid break down the hyaluronate molecules, thereby increasing the volume of synovial fluid and reducing its viscosity.



**FIGURE 1.** Formation of normal mucin precipitate in synovial fluid of the carpal joint in mixed-breed goats. Presence of firm coagulant in limpid solution.

**TABLE 2.** Cytological characteristics of synovial fluid in the carpal joint of mixed-breed goats

Characteristics	Mean	CI (95%)		SD	Minimum value	Maximum value
		LL	UL			
Global nucleated cell count (/μL)	48.02	37.83	61.95	24.47	22.50	112.50
Lymphocytes (/μL)	25.07	17.55	33.48	15.86	0.00	53.00
Large mononucleated cells (/μL)	16.31	10.67	23.21	12.63	0.00	44.00

CI = Confidence Interval; LL = Lower Limit; UL = Upper Limit, SD = Standard Deviation

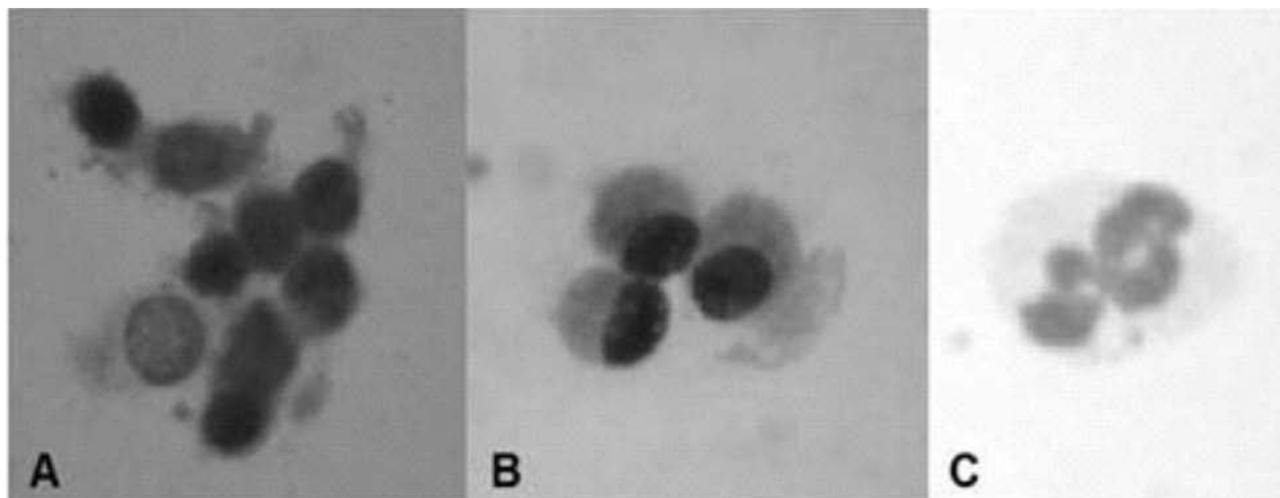
Regarding the cytological analysis, the mean global nucleated cell count was  $48.02 \pm 24.47$  cells/μL (Table 2). According to PARRY (1999), the nucleated cell count in normal synovial fluid varies according to the joint. A number of studies have established parameters for healthy joints, which generally have an overall count of less than 3000 cells/μL. According to BRANNAN & JERRARD (2006), the global cell count is an important exam for the classification of both inflammatory and non-inflammatory processes in joints. A study carried out by MARTINS et al. (2007) on the femoropatellar joint of horses found a correlation between viscosity and leukocyte count, in which the animals with no alteration in viscosity had leukocyte counts below 250 cells/μL and therefore had no joint inflammation. This corroborates the findings

of the present study regarding global white blood cell count and viscosity of the synovial fluid in the carpal joint of goats, in which 100% of the samples had global cell counts below 250 cells/μL.

In the differential leukocyte count (Figure 2), there was a predominance of lymphocytes over the other cell types encountered, representing 59.50% of the total, with values ranging from 0 to 53 cells/μL. The count of large mononucleated cells was 35.80%, ranging from 0 to 44 cells/μL (Table 2). The other leukocytes had low frequencies, such as neutrophils, which had constant values of 1 cell/μL. According to PARRY (1999), large mononucleated cells have a phagocytic potential and their origin is unknown; they may be derived from blood monocytes, tissue macrophages or cells from the synovial membrane.

The author found neutrophils at percentages below 5%, an absence of eosinophils, variable lymphocyte values (mean of 44%) and large mononucleated cells

in a considerably variable proportion. These findings are similar to those obtained in the present study on the carpal joint of goats.



**FIGURE 2.** Swab of synovial fluid from the carpal joint of mixed-breed goats. A. Agglomeration of lymphocytes and mononucleated cells; B. Undifferentiated mononucleated cells; C. Isolated neutrophil. (Rapid Panoptic). Barr = 5  $\mu$ m.

In inflammatory processes in joints, synovial fluid generally has a very poor quality mucin precipitate (FERNANDEZ et al., 1983) as well as an increased concentration of total proteins (VAN PELT, 1974) and a nucleated cell count between 4000 and 370,000 cells/ $\mu$ L (NELSON & COUTO, 1994). In degenerative joint disease, there is a slight increase in the volume of the synovial fluid and a reduction in its viscosity (WILKINS, 1993).

This research enhances data of characteristics of ovine synovial fluid. According to VAN PELT (1962), once normal physiochemical and cytological characteristics of the synovial fluid are known, these data may be employed to assess different types of arthritis as well as establish an early diagnosis and prognosis, determine the joint response to anti-arthritis and local and systemic anti-bacterial therapies, clarify the etiology and assist in the classification of diverse disease types.

### CONCLUSIONS

It is suggested that the synovial fluid of the carpal joint of adult mixed-breed goats must have as volume  $0.94 \pm 0.26$  mL; total protein  $1.95 \pm 0.87$

mg/dL and it must be colorless, limpid, with firm mucin precipitate in limpid solution. Cytological characteristics as number of global nucleated cells of  $48.02 \pm 24.47$  cells/ $\mu$ L; number of lymphocytes  $25.07 \pm 15.86$  cells/ $\mu$ L and large mononucleated cells  $16.31 \pm 12.63$  cells/ $\mu$ L may be considered normal for the synovial fluid of the carpal joint of this specie.

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Protocolado em: 22 fev. 2009. Aceito em: 7 set. 2009.