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Methods and Errors in Measurements of Synovial Fluid Volume in Stifles with Low Volume and High Viscosity Synovial Fluid. An Experimental Study in Goats

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Rørvik, A.M.: Methods and errors in measurements of synovial fluid volume in stifles with low volume and high viscosity venovial fluid. An experimental study in goats. Acta vet. scand. 1995, 36, 213-222. – Synovial fluid (SF) volume was calculated using various methods in the stifles of goats, in which the cranial cruciate ligament had been transected on one side. Measurements were performed prior to surgery and again 4, 8, and 18 weeks following surgery, by measuring the dilution of an injected radioactive tracer diluted by the SF. Later, 7 months following surgery, SF volume measurements using simple arthrocentesis were performed on stifles in 9 of the goats, and the SF that could not be aspirated, was calculated using 2 indirect methods simultaneously on identical fluids in 3 of these goats. SF was also collected directly during staged arthrotomy of the stifles in 4 goats.

There were conflicting results between methods, but the resulting calculated SF volumes seemed to be larger in the operated stifles compared to the controls for all the methods at about the same degree. The 2 indirect methods used to calculate the fluid remaining in the joints following arthrocentesis gave disparate volume calculations. The experiments revealed sources of error in all methods. Direct methods failed to acquire the total fluid volume, and indirect methods were subject to improper mixing and escape of the injected fluid or synovial fluid or both. It was concluded that none of the methods could be used to measure the "true" volume of SF, if such a concept exists and can be defined. None of the methods were considered reliable to compare volumes in different type of joints containing this type of fluid. It was, however, concluded that all the methods gave indication of increased SF volume present on a relative basis when paired joints were compared.

volume calculation; RISA; joint fluid; animal model; markers.

Introduction

Information about a disease process in a joint might in near future be obtained by examining and identifying various compounds (markers) released into the synovial fluid (SF). The analytical methods used to detect and measure the concentration of various substances in SF are highly sophisticated and accurate. Assessment of the activity of a disease process, (eg. the rate or degree of cartilage degradation) requires quantification of the markers *(Heinegård & Saxne* 1991). According to *Levick* (1990), the SF concentration of a marker cannot be accepted as a direct quantitative measure of the marker-release rate.

The volume of SF directly influences the concentration of a marker of joint disease by dilution, and also affects the rate of clearance from the joint *(Levick* 1990). *Page-Thomas et al.* (1987) found that the removal rate (or half life) of a marker was about the same in diseased joints containing large amounts of fluid compared to normal joints. This indicates that the amount of fluid cleared from the joint (clearance) is much higher in a joint containing a larger volume of SF *(Levick & Thompson* 1988). The concentration of a marker in SF may increase during the disease process, but using the concentration of the marker will greatly underestimate the amount of marker produced and liberated into the SF if the SF volume is increased *(Heinegård et al.* 1985). Thus, SF volume calculations are crucial when the liberation of a marker of joint disease into the SF are estimated. The volume may, however, not be regarded as a true measurement of the turnover of fluid in the joint.

Several methods for the calculation of SF volumes have been described, but no method has emerged as a reliable standard *(Simkin & Benedict* 1990). A frequently used method is based on the constant rate of elimination from the SF of an injected radioactive tracer. The tracer is eliminated from the joint by first order kinetics *(Kety* 1949). The log of the radioactive concentration in the SF is linear versus time after injection, so that the concentration at time zero can be extrapolated, and thereby the SF volume can be calculated. Radioactivity in a joint can be measured either by measuring SF samples or by using external radioactivity counts *(Ekman et al.* 1981, *Rekonen et al.* 1973, *Rodnan & Maclachlan* 1960, *Simkin & Benedict* 1990, *Wallis et al.* 1985, *Wallis et al.* 1987). At least 2 measurements of radioactivity taken with an interval of several hours from first to last measurement following injection depending on joint and tracer are required when using this method for SF volume calculation. This makes the method less practical on ordinary patients than the dilution methods described in this manuscript, but it may be the best method to estimate the "true" volume of SF in a joint. The aim of this study was to describe more convenient methods for estimation of synovial fluid volume and identify some of the errors of using these techniques in joints with small amounts of high viscosity fluid, and thus recognize pitfalls when markers of joint disease are quantified in synovial fluid.

Materials and methods

Methods for SF volume calculation used

The aspiration-method (ASP-method) is the direct measurement of as much SF as is possible to aspirate from the joint lumen using needle and syringe as described by *van Pelt* (1962).

The arthrotomy-method (ARTH-method) is the direct measurement of the SF using collection of SF by suction and wiping during a staged arthrotomy procedure as described by *Knox et al.* (1988).

The tracer-method (TR-method) is a "dilution method", where the SF volume was calculated from the dilution of an injected tracer, which was injected into the joint and diluted by the SF, as described by *Rekonen et al.* (1973).

The proteoglycan-method (PG-method) is also a "dilution method" where the volume of SF in the joint is calculated from the dilution of SF by fluid injected into the joint cavity, originally described by *Geborek et al.* (1988) using albumin as marker instead of PG.

The SF samples were frozen at –28°C and kept frozen until analysis. The concentration of PG in the samples was determined by an enzyme linked immunosorbent assay (ELISA) technique. The analyses were performed by

collaborators at the Department of Physiological Chemistry, University of Lund, Sweden, using an ELISA specially made for goats. Antibodies were produced in calves from goat proteoglycan preparations extracted from shoulder joint articular cartilage harvested from a goat at the same age as the experimental animals. The ELISA was originally described measuring PG on dog SF by *Heinegård et al.* (1985), later described used on human SF in more detail by *Saxne et al.* (1986).

Experimental animals

The 13 goats used in these experiments were young (age matched), healthy, castrated male goats. Transection of the cranial cruciate ligament in the left stifle was performed when the goats were about 6 months of age.

Experimental protocol

The SF volumes were calculated in both stifles of the 13 goats before surgery and again at 4 weeks, 8 weeks, and 18 weeks following surgery using the *TR-Method.* The goats were sedated using medetomidine hydrochloride (Domitor®) 20 μg/kg intravenously. Arthrocentesis was performed using sterile technique and minimum trauma. Stifles were punctured from the proximo-lateral side between the patella and the lateral patellar trochlea. When shoulder joint arthrocentesis was performed, the needle was introduced on the dorso-cranial aspect, under the tendon of the biceps brachii muscle. Ten ml saline, containing RISA (radioactive isotope-marked serum albumin), was injected slowly into the joint cavity using a glass syringe and gentle "one finger" pressure applied to the plunger. The needle was removed, and the joint was passively manipulated by extension and flexion 20 times in 20 seconds before arthrocentesis was performed. Injection and arthrocentesis was performed using 21G×1.5″ needles. RISA containing 48 μCi/mg of ^{125}I-marked goat serum albumin was produced and provided by The Hormone Laboratory, Aker Hospital, Oslo. The RISA used for injection was freshly prepared in 0.9% saline to which 0.2% unlabelled albumin had been added as a competitor to reduce the effect adherence of the radio-labelled albumin to the walls of tubes and syringes.

The concentration of RISA in fluid was measured by γ-counting using automatic well scintigraphy[1]. Formula (1) expressed the SF volume (V_{SF}):

$$V_{SF} = \frac{V_{INJ}(C_{INJ} - C_{DIL})}{C_{DIL}} \quad (1)$$

where C_{INJ} was the tracer concentration in the injected fluid, C_{DIL} was the concentration in the recovered fluid and the volume of injected RISA solution was V_{INJ} (10 ml). The tracer concentration in the injected RISA solution (C_{INJ}) was adjusted to be between 3000 to 20,000 cpm/ml before injection and established using the mean number of counts from 5 one ml samples.

As a control of the mixing procedure within the joint when the indirect methods were used, the fluid was aspirated and kept in separate fractions (fractionated aspiration) following injection. The SF volumes may be calculated from every fraction of fluid aspirated from the joint and were calculated using the first 1 ml and the last 1 ml of recovered fluid, and the acquired volumes were compared. This was done in the stifle and shoulder joints of 3 goats (6 stifles and 6 shoulders). Standard procedures of the TR-method were followed

[1] Packard Auto-Gamma® 5650, Packard Instrument Company INC, Downers Grove, ILL 60515.

regarding injection and manipulation. The same trials were also performed using 2 ml and 5 ml injected volumes instead of 10 ml on stifles in each of 2 goats, and the volumes were calculated from the first and last 0.5 ml fractions.

Seven months following surgery, the SF volume was estimated using simple arthrocentesis with a disposable needle and a 2 ml plastic syringe to evacuate as much SF as possible *(ASP-method)* from the stifles of 9 goats. The needle and the syringe were weighed before and after arthrocentesis, and the SF volume was defined as the difference in weight (V_{ASP}). Preparation of the joint was the same as used in the TR-method.

The SF which remained in the joint (V_{REST}) and could not be aspirated was calculated using 2 indirect methods simultaneously on identical fluids on 3 of these 9 goats (6 stifles) for comparative purposes. One of the methods was the TR-method previously described. The other method was the PG-method. Proteoglycan (PG) concentration was analyzed in SF before and after fluid injection. The following formula (2) expressed the SF volume prior to injection of fluid, after a sample had been aspirated from the joint:

$$V_{REST} = \frac{PG_{DIL} \times V_{INJ}}{PG_{ASP} - PG_{DIL}} \qquad (2)$$

V_{REST} is the remaining SF in the joint following aspiration, V_{INJ} is volume injected fluid (in this case 10 ml of RISA solution), PG_{ASP} is the concentration of PG in SF before injection (as in V_{ASP} and V_{REST}) and PG_{DIL} is the PG concentration in the diluted SF following injection. The total SF volume could be expressed and calculated: $V_{SF} = V_{ASP} + V_{REST}$. Calculation of V_{REST} using the TR-Method and PG-method simultaneously from identical samples was possible because the injected fluid V_{INJ} was a RISA solution with known radioactivity C_{INJ}, giving radioactivity in the joint C_{DIL}.

$$V_{REST} = \frac{PG_{DIL} \times V_{INJ}}{PG_{ASP} - PG_{DIL}} \approx \frac{V_{INJ}(C_{INJ} - C_{DIL})}{C_{DIL}} \qquad (3)$$

Formula (3) expresses the expected result if the injection and mixture procedures can be made without creating significant errors in calculations of V_{REST}.

Eight months following operation, the SF volume was estimated using collection of SF by suction during a staged arthrotomy procedure (ARTH-method) in 4 goats. This method required the animal to be killed before or immediately after the collection procedure. The goat was sedated as in the other procedures, and epidural anaesthesia was administrated using 5 ml lidocain hydrochloride 2% (Xylocain® adrenalin) injected into the lumbosacral foramen. A thin vinyl tube leading into a collecting tube connected to a vacuum machine was used to aspirate SF. Suction was begun through a small hole in the joint capsule and continued during the staged surgical exposure of the joint structures. Haemorrhage was controlled by the use of thermo-surgery. A highly absorbent material (Gaasdeppers HA0252) was used to wipe surfaces and collect the SF that remained after completed suction. The collection tube with the vinyl tube and the absorbing material were weighed before and after the fluid collection, and the volume of SF was defined as the difference in weight.

An attempt to estimate the amount of fluid lost during injection, manipulation, and recovery procedures was made by injecting the shoulder joints in 3 goats with 10 ml 0.9 % saline. The joints were passively moved according to the TR-method. Then as much injected

Table 1. Mean synovial fluid volumes ± standard deviation (SD) in operated (+) and contralateral control stifles (knees) of 13 goats calculated by the TR-method (++) and mean difference in SF volume ± standard error of means (SEM) by paired observations (paired obs.). Volume was calculated prior to operation and again at 4, 8 and 18 weeks following operation. Thirteen samples excluded.

Time	Operated stifles SF volumes (ml) Mean ± SD (n)	Control stifles SF volumes (ml) Mean ± SD (n)	Paired observations Difference in ml Mean ± SEM (n)
Before surgery	4.0 ± 1.7 (9)	4.6 ± 2.4 (10)	0.3 ± 0.63 (8)
4 weeks after surgery	7.5 ± 2.2 (13)	5.1 ± 1.6 (12)	2.6 ± 0.59 (12)
8 weeks after surgery	4.7 ± 2.1 (11)	3.0 ± 1.2 (12)	1.8 ± 0.49 (11)
18 weeks after surgery	5.9 ± 2.7 (12)	4.2 ± 2.4 (11)	1.9 ± 0.31 (11)

(+): Operation: transection of the cranial cruciate ligament in left stifles.
(++): Volume calculated from degree of dilution of injected RISA solution by the SF.

fluid as possible was aspirated using a needle and syringe. The goat was killed and the shoulder joints dissected immediately. The remaining free fluid was collected from the opened joint.

Statistical methods

Student's "t" test for paired comparisons were used *(Sokal & Rohlf* 1981) to compare operated and control stifle SF volumes.

Criteria for exclusion of animals and samples

Animals were excluded if infection in the stifles was evident. SF samples were excluded (blind exclusion) if infection was suspected. Samples were also excluded if, following injection of fluid into the joint, only a small amount of fluid was recovered and this fluid contained significant quantity of an amorphous substance.

Results

The results of synovial fluid (SF) volume calculations from stifles of 13 goats using the TR-method are given in Table 1. The SF volume in the operated stifles was larger than the SF volume in contralateral, unoperated control stifles. Calculated on paired observations, the difference was present on all 3 calculations 4 weeks ($p<0.0005$), 8 weeks ($p<0.005$) and 18 weeks ($p<0.0005$) after surgery. The estimated SF volume varied considerably among joints and among calculations of the same joint.

When a small volume (2 ml or 5 ml) of RISA solution was used for performing the TR-method, and SF volume was calculated from the first and last fractions aspirated (fractionated aspiration), large differences in volume were calculated. Less variation was found between first and last aspirate following injection of a 10 ml volume. Despite a range of –20% to +17% of the calculated volumes, most of the calculations from first and last fractions were consistent following 10 ml injections, and the mean difference between these calculated volumes was –2.5 % (n = 12).

Results of SF volume estimation using maximum aspiration by arthrocentesis (ASP-method) in 9 goats 7 months following operation are given in Table 2. The mean difference ± SEM in SF volume between operated and control stifles by paired observations was 0.31 ± 0.05 ml (n = 8 paired observations).

Calculations of the residual SF (after evacuation of SF) using the TR-method and the PG-method simultaneously in the stifles of 3 of

Table 2. Synovial fluid volume estimations in operated (+) and control stifles calculated 7 months following operation using direct aspiration (ASP-method) in the stifles of 9 goats (18 joints, 1 missing) followed by calculations of the residual SF in the joint on 3 of the goats using 2 indirect methods (++) simultaneously.

Goat	Arthrocentesis (ASP-method)		Rest volume TR-method		Rest volume PG-method	
	Operated	Control	Operated	Control	Operated	Control
1	0.94	0.57				
2	0.51	0.11				
3	0.48	0.12				
4	0.39	0.21				
5	0.36					
6	0.90	0.64				
7	0.73	0.30	4.4	3.2	1.6	1.2
8	0.44	0.24	3.6	3.7	2.4	0.9
9	0.88	0.36	5.5	3.7	3.7	1.2
Mean	0.63	0.32	4.4	3.5	2.5	1.0
SD	± 0.24	± 0.20				

(+): Transection of cranial cruciate ligament was performed in the left stifle 7 months prior to measurements.
(++): The TR-method calculated dilution of injected fluid by the SF, and the PG-method calculated dilution of SF by injected fluid.

the goats are shown in Table 2. The residual SF being the object of calculations was identical for the 2 methods, however, the resulting calculated volumes were highly different for the 2 methods (Table 2).

The volumes of SF collected from stifles of 4 goats using the ARTH-method are shown in Table 3. There were differences between operated and control joints, however, the samples were few. In operated stifles the mean volume of SF was 2.6 (n = 3), and in controls the mean volume was 1.9 (n = 4).

When 10 ml saline was injected into shoulder joints and the joints were manipulated using the procedure described in the TR-method, only 7.5 ± 0.6 ml (n = 6) fluid could be aspirated from the joint. An additional 0.5-1.0 ml could be collected by arthrotomy performed immediately after the goats were killed.

Discussion

All the methods for the calculation of SF volume applied in this study found larger volumes in operated stifles than in the control stifles, but the calculated volumes differed between methods. The TR-method provided SF samples for eventual marker analysis, and this is an important issue. The procedure was rapid and easy to perform and did not seem to harm the joint. The TR-method was consid-

Table 3. Synovial fluids (SF) volume measured in operated (+) and control stifles of 4 goats using stepwise arthrotomy, while SF was collected (ARTH-method) 8 months following operation.

Goat	Operated stifle	Control stifle
10	4.9	1.7
11	1.4	0.8
12	1.8	1.2
13	-- (++)	2.0
Mean	2.7	1.9

(+): Transection of cranial cruciate ligament was performed in left stifle 8 months prior to calculations.
(++): Excluded due to suspected infection.

ered superior to the PG-method in these joints because the latter method required the collection of a sample of undiluted SF, which is not always easily accomplished. The major disadvantage of the TR-method was the use of a radioactive tracer and that access to scintigraphy was required. In joints where SF is easily available, the PG-method should be preferred using albumin or globulin as volume marker.

Using the aspiration-method (ASP-method) the aspirated volume should represent the amount of the free fluid available in the lumen of the joint, but there are considerable difficulties associated with complete drainage of the joint cavity *(Ekman et al.* 1981). Calculation of the amount of SF which remained in the joint cavity following evacuation by arthrocentesis gave useful information. Not only did it show that fluid remained in the joint although the joints had been "emptied", as suggested by *Ekman et al.* (1981). It also showed that the SF residual volume calculated using the TR-method and the PG-method gave highly different estimated volume when identical SF fluid was the object of calculation. This may be explained by the effect of the physiological and morphological situation in the joint cavity on these measurements.

Firstly, the cellular layer of the synovial membrane covers only parts of the surface, and it has no basal membrane *(Knight & Levick* 1984, *Wallis et al.* 1987). The SF volume incorporates fluid within the interstitium of the synovial tissue. When using indirect methods of measurement, the tracers also mix with this "hidden" fluid. The tracers probably invade the interstitium to a varying degree depending on the nature, size, and polarisation of the tracer used *(Cochrane et al.* 1965), and the calculated SF volume may vary accordingly *(Simkin & Benedict* 1990).

Secondly, the injected fluid or tracer has to be evenly mixed with the SF. According to *Wallis et al.* (1985) RISA has ideal behaviour in SF and other body fluids. It does not aggregate or precipitate and does not initiate phagocytic activity, but mixes evenly with body fluids and behaves like ordinary albumin in the tissues. Other tracers may give biased results due to uneven mixture, phagocytosis or adherence to synovial tissue or cartilage. In the goat joints, mixing injected fluid with SF was an apparent source of error. When a smaller volume of RISA solution (2 or 5 ml) was injected performing the TR-method and fractionated aspiration was used, large differences in volume were calculated from first and last fractions aspirated. This was considered to be the result of poor mixing of injected fluid with SF. Less variation was found between volume calculations from first and last aspirate following injection of a 10 ml volume.

Thirdly, the indirect methods of SF volume calculations are based on the concept that the joint lumen is a well defined space that does not leak during injection and mixing. Using a larger volume of injected fluid reduced an error by improving the mixing between injected fluid and SF. Another error may, however, have been introduced as was demonstrated in these experiments when injected fluid disappeared from the shoulder joints. Increased pressure in the joint during injection and mixing may result in fluid loss. Synovial fluid is normally drained from the joint through the lymphatic system as a "bulk flow", and for the macro-molecules this is the main elimination route *(Wallis et al.* 1987). Undiluted SF and injected fluid may have been forced out through the lymphatic drainage during the manipulation of the joint, which in either case would have opposite effect on the calculated SF volume by the 2 diluting methods (TR and PG) (see Formula (3)). The SF volume in stifle

joints is small relative to the area of cartilage and synovial membrane articular surfaces, and thus small relative to the potential volume of the joint. At least 50 ml fluid could be injected into a human knee without increasing the internal pressure in the joint, and distention could not be clinically recognized following this injection, although the amount of SF is normally not exceeding 5 ml in humans *(Geborek et al.* 1988). Ten ml injection in the stifles of the goats could not be clinically recognized as a distention in the present study. The pressure in the joint was probably also not increased in the goat stifles during injection. The pressure may still have exceeded normal values following injection of 10 ml saline in the shoulders, and this distention may explain some of the loss of fluid found following this procedure in the shoulders of the goats. Also the extensive movement of the stifles to provide mixing of injected fluid with synovial fluid could have resulted in fluid elimination from the cavity of the injected joints. Movement of the patella for 2 minutes as a mixing procedure *(Geborek et al.* 1988) might be more secure.

The ARTH-method (staged arthrotomy) was used in order to acquire the "true" SF volume, to evaluate the other methods. However, it was quickly recognized to be subject to a serious error. The volume measurements obtained by cautious versus vigorous wiping were quite different. This method was complex, difficult, and failed to fulfil the intended purpose. Therefore, the intention to use this method on all 13 goats was abandoned.

In several cases, aspiration of fluid from joints following injection was difficult or impossible to accomplish because of a gelatinous, colourless mass that did not sediment during 2200 G centrifugation for 30 min. The gelatinous mass was thought to be hyaluronan, although it was not chemically analyzed. This presented more of a problem in normal and control joints than in operated joints. Samples containing large quantity of this material were shown to have correspondingly decreased radioactivity in pilot studies on normal joints. The clots were easy to see in the tubes, although failure to detect this material and exclude the respective sample might be a source of error in indirect SF volume calculations.

The present study indicates that suppositions regarding indirect methods of SF volume are not all true for joints containing small amounts of SF with high viscosity. Errors in SF volume estimation using indirect methods are probably less significant in simple, large joints containing large volumes of fluid, such as in the large joints of the horse. In these joints, small volumes of tracer solutions can be injected without the problem of improper mixing or increased pressure in the joint, and a fluid volume equal to the volume of tracer can easily be evacuated prior to injection. Simultaneous volume calculations using 2 methods may be performed also in these joints as a control to errors (Formula (3)) in scientific studies. It is probable that the degree of complexity of the joint and low SF volume increases the degree of erroneous calculations of SF volumes, but this needs further study.

Faced with possible errors associated with SF volume measurement, lavage of the joint to collect SF might be preferred. As much as possible of SF is evacuated from the joint using arthrocentesis. Then a volume of saline much larger than the expected SF volume is aspirated into a syringe. A part of this, not causing elevated pressure in the joint, is injected into the joint cavity, and the resulting mixture is aspirated back into the syringe. Then the mixture in the syringe is re-injected into the joint. This procedure is repeated several times, perhaps interrupted by some procedure of movement of the joint and shaking

of the syringe, until it is assumed that the concentration of marker in the syringe has reached its maximum. Finally, the initially evacuated SF is aspirated into the syringe. The concentration of marker in the syringe, after mixing, is then a direct relative measurement of the total amount of marker in the joint cavity. The total amount of marker, that was present in the joint cavity, is this concentration times the dilution volume (the sum of the initial volume of saline in the syringe and the amount of SF initially evacuated). Calculations of SF volume and the error of using SF concentration of a marker as a measurement of marker production are avoided. However, some of the same errors found in the present study on SF volume calculation may also be present using the lavage procedure.

It was concluded that all the methods used in this study to estimate SF volume were encumbered with errors, and that the "true" SF volume in these joints was still unknown, if such a volume can be defined and measured. Despite the errors inherent to the measurement of SF volume, as were outlined in this study, a relative difference in SF volumes of paired joints could be estimated. Marker concentrations could roughly be corrected according to the relative SF volumes found by all the methods outlined in this study, although the accuracy of the figures was unknown. Synovial fluid analysis seems to become an important way to judge the health of a joint in near future. The sources of error when SF is collected for diagnostic purposes need further investigation.

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Sammendrag

Noen målemetoder og feilkilder ved måling av leddvæskevolum i kneledd med lite leddvæske med høg viskositet. Et eksperimentelt studium på geit.

Volumet av leddvæske (LV) ble beregnet flere ganger i kneledd på 13 geiter ut fra den fortynning som oppsto når væske med kjent radioaktivitet ble injisert og fortynnet av leddvæsken. Det craniale korsbånd ble kuttet ved operasjon i venstre kne da geitene var ca. 6 mnd. gamle. Målingene ble utført på geitene før operasjonen og igjen 4, 8 and 18 uker etter operasjon. Senere, ca. 7 måneder etter operasjon, ble LV-volumet målt direkte ved arthrocentese i kneledd på 9 av geitene, og den leddvæske som ble tilbake i leddet og ikke kunne aspireres, ble målt simultant ved hjelp av to indirekte metoder på 3 av geitene. LV-volum ble også målt i 8 kneledd på 4 geiter ved at leddet ble åpnet etappevis og LV samlet opp og veiet.

Alle metodene som ble prøvet, ga relativt like stor forskjell i LV-volum mellom opererte kneledd og kontroller, men volumene som ble funnet varierte sterkt metodene imellom. Mye LV forble i leddene etter arthrocentese, og de to metoder som ble brukt til å kalkulere denne væskemengden ga vidt forskjellig resultat. Dette ble forklart ved at de viktige feilkilder til indirekte LV-målinger virker motsatt på resultatet ved bruk av de to metodene. Dette kan brukes til å kontrollere feilkilder ved bruk av indirekte målinger av LV-volum. Det ble konkludert med at alle metodene kunne brukes til å sammenligne leddvolumer i parledd på relativ basis, selv når LV-volumet var lite med høg grad av viskositet, men ingen av metodene ga et korrekt mål for LV-volumet i leddene. Metodene kan dermed ikke brukes til å sammenligne LV-volumer i forskjellig utformede ledd med denne typen LV. Feilkildene var store, og tap av injisert væske og dårlig blanding av LV med injisert væske kan være en stor feilkilde når indirekte måling av LV-vulum blir utført. Forholdene i leddhulen og feilkildene ved uttak av leddvæskeprøver for diagnostiske analyser trenger ytterligere utredning, og videre forskning er viktig, fordi analyser av leddvæske kan bli en viktig del av diagnostikken på ledd i nær framtid.

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