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Effect of Age and Abomasal Puncture on Peritoneal Fluid, Hematology, and Serum Biochemical Analyses in Young Calves

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The goals of this study were to evaluate techniques for collection of peritoneal fluid from calves, establish reference ranges for fibrinogen in peritoneal fluid during the 1st month of life, and determine if abomasal puncture would alter peritoneal fluid or hematologic variables. Twenty-two healthy Holstein calves underwent 3 peritoneal fluid collections on day 1, day 15, and day 30 of age. Fibrinogen concentration in peritoneal fluid was 0.20 g/dL and 0.10 g/dL ($P < .05$) for day 1 and day 30, respectively, and 0.10 at day 15 ($P > .05$) for calves without abomasal puncture. Plasma fibrinogen concentration was 0.60 g/dL and 0.70 g/dL ($P < .05$) for days 15 and 30, respectively, in calves without abomasal puncture. There were no significant differences ($P \leq .05$) in peritoneal fluid and peripheral blood total protein and fibrinogen concentrations, specific gravity, total and differential cell count, or erythrocyte counts between calves with or without abomasal puncture. We concluded that the reference ranges established for fibrinogen and total protein concentration are important for accurate evaluation of peritoneal fluid in calves for further comparison with similar-aged animals with gastrointestinal-tract or abdominal-cavity disease. Additionally, accidental abomasal puncture does not alter values of fibrinogen, total protein, and nucleated cell count in peritoneal fluid and does not cause apparent clinical abnormalities.

Key words: Abdominal paracentesis; Bovine; Fibrinogen; Total protein.

Peritoneal fluid analysis can be of value in the diagnostic evaluation of disorders of the abdominal cavity in calves. It is necessary to use a safe and effective technique for peritoneal fluid collection both to maximize the chances of obtaining a quality sample and to minimize the likelihood of complications.

Normal peritoneal fluid provides lubrication for the movement of abdominal organs and apposed peritoneal surfaces.[1] A normal animal has no more than 1 mL of peritoneal fluid/kg of body weight,[2] and in acute severe peritonitis, the inflammatory process may induce a net flow of liters.[3]

Abdominocentesis has been used successfully in many species to aid in the diagnosis of abdominal-cavity diseases.[4] It can be used to assess the volume of peritoneal fluid, cellularity and protein concentration, thereby giving an indication of the extent of inflammatory or neoplastic changes in the peritoneal cavity.[5] Additionally, cytologic evaluation of the fluid may allow determination of a more definitive diagnosis.[6] Changes in the constituents of peritoneal fluid have been described for left-sided displacement of the abomasum, traumatic reticuloperitonitis, septic peritonitis, intra-abdominal neoplasia, after laparoscopic surgery, and after exploratory celiotomy and omentopexy in adult cattle.[7]

Peritoneal fluid values for calves are different from values of adult cows, except for neutrophil and lymphocyte counts, total protein concentration, and specific gravity.[4]

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Calves tended to have more neutrophils and lymphocytes and lower total protein concentration in peritoneal fluid than did cows.[4] Thus, reference ranges established for peritoneal fluid constituents of clinically normal adult cattle may not be appropriate guidelines for interpretation of peritoneal fluid in young calves.[4]

Analysis of peritoneal fluid collection from 10 healthy, young, male Holstein calves using a new collection technique revealed findings indicating that utilizing reference ranges for adult cattle or even older calves may result in erroneous interpretations when evaluating peritoneal fluid from younger calves.[8]

The goals of this study were to evaluate the technique for collection of peritoneal fluid from calves as proposed by Burton et al,[8] establish values of fibrinogen concentration in peritoneal fluid during the 1st month of life, and determine if an abomasal puncture would alter peritoneal fluid or hematology findings.

Materials and Methods

Animals

Twenty-two healthy Holstein calves (9 female and 13 male) were included in this study. After colostrum ingestion, the calves were separated from dams and fed 4 L of milk, divided into 2 feedings daily. Calves were kept on pasture during the day and housed during the night. Water, coast-cross hay and concentrate (from the 7th day of age on) were free-choice available. The experimental protocol was approved by the local Animal Care and Use Committee.

Clinical Monitoring

Daily physical examination, including evaluation of the umbilical stalk and rectal temperature, was performed on every calf every day during the 30 days of this study. No animal showed visual or palpable abnormality, such as heat or increase in size or firmness of the umbilical stalk before the onset or during the study. The internal stalk was externally evaluated by digital palpation, looking for signs of pain or local sensitivity evoked by the manipulation. Lung auscultation and heart and respiratory rates were performed every day to eliminate the occurrence of pneumonia. Joints were palpated for signs of heating, pain, or swelling during the whole study. One animal showed signs of

inappetence, apathy, increased respiratory rate and pulmonary crackles, fever, and coughing 7 days after the 1st peritoneal fluid sampling, and its data were excluded from the study.

When accidental abomasal puncture occurred, calves were examined for signs of fever, septicemia, abdominal pain, or abdominal wall sensitivity at the site of the puncture until 2 weeks of the 3rd and last sampling.

Abdominocentesis and Sampling Times

Each calf underwent 3 peritoneal fluid collections, the 1st on day 1, the 2nd on day 15, and the 3rd on day 30 of age. For the abdominocentesis, calves were sedated with diazepam[a] (0.05 mg/kg of body weight, IV) and xylazine[b] (0.05 mg/kg of body weight, IM) and positioned in left lateral recumbency with the right hind leg pulled dorsally and caudally. The hair was clipped over an area approximately 15 cm in diameter at a position slightly dorsal and caudal to the umbilicus. Hair was also clipped over an area approximately 10–15 cm in diameter at a 2nd site in the center of the right inguinal region (10 cm caudal to the umbilical scar and 10 cm laterally off the midline toward the right inguinal region). The skin at both sites was prepared aseptically. The 1st site for abdominocentesis (4–5 cm dorsally off the umbilical scar toward the right abdominal wall) was injected subcutaneously with 1 mL of 2% lidocaine.[c] Then a 14-gauge, 5-cm needle[d] (assembled with a polyurethane catheter inside a sterile nylon sleeve) was inserted into the anesthetized site, aiming slightly caudally and toward the midline, keeping the needle parallel to the inner abdominal wall once the abdominal cavity was penetrated. A polyurethane catheter[d] (30.4 cm) was passed through the needle and a 10-mL sterile syringe was attached to the catheter and gentle aspiration was used to obtain peritoneal fluid. While applying gentle aspiration on the syringe, the catheter was moved gently in its full length and in all directions (dorsally, caudally, or ventrally) within the abdominal cavity to maximize fluid retrieval. When the sample volume obtained from the 1st site was insufficient or if it was not possible to collect any fluid or when the abomasum was accidentally punctured, aspiration was performed at the center of the previously prepared inguinal site (2nd site), using the same technique described above. Care was taken to maintain the catheter tip parallel to the inner abdominal wall from the time of insertion until removal of the needle.

Peritoneal Fluid Analysis

After collection, the peritoneal fluid was transferred into a tube containing ethylenediaminetetraacetic acid (EDTA)[e] and was immediately sent to the laboratory for cytology and biochemical analyses. Peritoneal fluid assessment included physical characteristics (color and turbidity) and laboratory measurements (total and differential cell counts, total protein concentration, fibrinogen concentration, and specific gravity).

Peritoneal fluid total protein concentrations and specific gravity were determined by a light refractometer,[f] using the supernatant obtained after centrifugation at $200 \times g$ for 5 minutes. Peritoneal fluid fibrinogen concentration was determined by the heat-precipitation-refractometry method.[9]

Total nucleated cells were counted by means of a Neubauer chamber. Cytologic evaluation and differential determination were performed on slides stained with Leishman stain. Differential counting of 100 cells was performed with nucleated cells classified into 1 of 3 categories: polymorphonuclear cells (neutrophils, basophils, and eosinophils), mesothelial cells/macrophages, and lymphocytes.

Peripheral Blood Analyses

Each time peritoneal fluid was collected, blood was obtained by jugular venipuncture and placed into sterile tubes containing EDTA.[e] Hemograms were counted by means of a Neubauer chamber and plasma total protein concentrations were determined by a light refractometer,[f] using the supernatant obtained after centrifugation at $200 \times g$ for 5 minutes. Plasma fibrinogen concentration was determined as described for peritoneal fluid.

Statistical Analyses

A computer software program[g] was used for statistical calculations. Data from hematologic and peritoneal fluid analyses were summarized, and median and range were reported for each of the 3 sampling periods. Multiple comparisons between days were performed by the Kruskal-Wallis 1-way analysis of variance, which included all calves with or without abomasal puncture. To evaluate if the accidental abomasal puncture would interfere with data obtained, comparisons between calves with and without abomasal puncture were performed using the Wilcoxon rank sum test.[h] Differences between medians for each of the 3 sampling periods were compared using the Dunn test, with $P \leq .05$ considered significant.

Results

Peritoneal Fluid Collection Technique

The combination of diazepam and xylazine was effective to facilitate collection of peritoneal fluid from prechosen sites.

The abdominal paracentesis technique developed by others[8] was safe for calves and successful in obtaining the peritoneal fluid and making its analysis in the majority of samples possible. However, it was noticed that the efficacy of collection and the volume of fluid sampled increased when it was performed in calves older than 15 days of age and with the experience of the veterinarian that performed the technique.

Fluid was obtained at 12 samplings in 1-day-old calves, with 9 successful collections at the 1st site and 3 at the 2nd site. The volume of peritoneal fluid collected was less than 1 mL.

Fluid was obtained at 22 samplings in 15-day-old calves, with 8 successful collections at the 1st site and 10 at the 2nd site, whereas in another 4 animals, fluid was collected at both sites because the volume recovered at the 1st site was not sufficient to perform the laboratory analyses. The volume collected was approximately 1.5 mL.

Fluid was obtained at 21 samplings in calves 30 days old; of the 21, successful collection of fluid was obtained, with 17 being recovered at the 1st site and 4 being collected at the 2nd site. The volume collected was 3 mL.

Although not intended, puncture of the abomasum occurred in 4 animals at day 1, 7 at day 15, and 4 at day 30.

While applying gentle aspiration on the syringe, the fluid collected had a yellowish aspect with large, white clots. The volume obtained was 15 mL. The catheter and the needle were immediately withdrawn from the abdominal cavity and the fluid was inserted into a tube (vacutainer) with EDTA. The odor was characteristic of that of digested milk. Then the 2nd site was aseptically prepared and a new catheter was used to perform the aspiration at the center of the right inguinal region, using identical local anesthesia as previously described. Abomasal puncture appeared to be associated with performing the collection technique in calves that had been recently fed. No clinical abnormalities were noticed to develop subsequent to these accidental punctures. The volume of fluid collected in these animals was approximately 1.5 mL.

Table 1. Peritoneal fluid data from young Holstein calves.

Analyte	Day	n	Value	
			Median	Range (min, max)
Fibrinogen (g/dL)	1	10	0.10[b]	(0.10, 0.50)
	15	18	0.10	(0.10, 0.50)
	30	21	0.20[a]	(0.10, 0.60)
TP (g/dL)	1	11	4.30	(1.80, 6.40)
	15	22	3.40	(1.60, 6.40)
	30	21	3.20	(1.40, 5.60)
Specific gravity	1	11	1.032	(1.016, 1.040)
	15	22	1.026	(1.015, 1.040)
	30	21	1.026	(1.016, 1.038)
NCC (cells/μL)	1	9	1,850	(43, 7,750)
	15	21	3,150	(350, 13,050)
	30	21	4,200	(260, 13.500)
PMN cells (%)	1	10	24.0	(17, 60)
	15	18	24.5	(5, 79)
	30	17	36.0	(9, 66)
Mesothelial/mononuclear cells (%)	1	10	72.0	(32, 77)
	15	18	69.0	(20, 90)
	30	17	58.0	(28, 89)
Lymphocytes (%)	1	10	4.0	(0, 11)
	15	18	5.0	(0, 19)
	30	17	6.0	(2, 21)
RBC (cells/μL)	1	10	8,750	(750, 114,000)
	15	20	22,500	(250, 260,000)
	30	21	4,775	(1,700, 59,250)

n, number of samplings; TP, total protein; NCC, nucleated cell count; PMN, polymorphonuclear cells; RBC, red blood cells count. Values for each analyte category followed by different letters are statistically different (Kruskal-Wallis for comparisons among 3 groups, $P < .05$).

No increase in peritoneal fluid volume was observed after any accident nor was the urinary bladder punctured when performing the collection at the 2nd site. No bacteria or food were observed on smears prepared with the fluid obtained from the 2nd site of sampling.

Peritoneal Fluid Findings

Peritoneal fluid color varied from pale yellow to very pale red. All samples were slightly cloudy. Fibrinogen concentration in peritoneal fluid was significantly increased in 30-day-old calves compared with 1-day-old calves but was not different from 15-day-old calves without abomasal puncture (Table 1). However, fibrinogen concentration was not different between calves with or without abomasum puncture. No significant differences were observed in total protein, specific gravity, total nucleated cell counts, polymorphonuclear cell count, mesothelial/macrophage cell count, lymphocyte count, and erythrocyte count among groups and between calves with or without abomasal puncture (Table 2). In this study, mesothelial/macrophage cells were the predominant cell type present in the peritoneal fluid (Table 1).

Erythrocytes were observed in peritoneal fluid, but there was no significant difference between mean RBC counts in peritoneal fluid over the 3 sampling times (Table 1) and between calves with or without abomasal puncture (Table 2).

Peripheral Blood Findings

Plasma fibrinogen concentration was significantly increased ($P < .05$) for days 15 and 30 in calves without abomasal puncture (Table 3).

Complete blood counts were performed to follow-up animals from all sampling times. There was no significant increase in total nucleated cell count, plasma total protein concentration, or plasma fibrinogen concentration in calves with abomasal puncture compared with calves without puncture in the same group over time (Table 4). Because there were no peritoneal fluid alterations observed over time for the calves with abomasal puncture, they were evaluated clinically for only 2 weeks after the end of the study. There were no apparent clinical alterations in calves subsequent to the puncture.

Discussion

Compared with adult cattle, healthy calves are more difficult to restrain while standing.[8] The technique used for sedation of these animals appeared to be safe, efficient, and was relatively inexpensive, making it useful to perform other procedures in calves. No anesthetic accidents or complications were observed. Sampling with the calves sedated and in lateral recumbency minimizes the probability of accidents,[8] such as, for example, perforation of bowels or physical trauma during the restraint.[10] It is advisable to

Table 2. Peritoneal fluid data from young Holstein calves with or without abomasal puncture.

Analyte	Abomasal punction			
	Yes		No	
	n	Value Median (min, max)	n	Value Median (min, max)
Fibrinogen (g/dL)	10	0.20 (0.1, 0.6)	39	0.10 (0.10, 0.50)
TP (g/dL)	11	3.0 (1.6, 6.4)	43	3.9 (1.4, 6.2)
Specific gravity	11	1.026 (1.015, 1.040)	43	1.029 (1.016, 1.040)
NCC (cells/μL)	11	3,300 (650, 13,050)	40	2,850 (43, 13,500)
PMN cells (%)	10	34.5 (7, 61)	35	25 (5, 79)
Mesothelial/mononuclear cells (%)	10	61.5 (33, 88)	35	68 (20, 90)
Lymphocytes (%)	10	6.5 (0, 21)	35	5.0 (0, 19)
RBC (cells/μL)	11	6,100 (250, 114,000)	40	14,491 (750, 260,000)

n, number of samplings; TP, total protein; NCC, nucleated cell count; PMN, polymorphonuclear cells; RBC, red blood cells count. Values for each analyte category did not differ at 5% (Wilcoxon's rank sum test for comparisons between animals with and without abomasal punction, $P > .05$).

avoid the use of this technique within the 1st hour after feeding since the abomasum can be accidentally punctured due to its distention. When accidental abomasal puncture did occur in calves of this study, there were no clinical or physical abnormalities or growth delay observed through 6 months after the collection of the 3rd and final peritoneal fluid sample. Calves that had puncture of the abomasum had RBC, total and differential white cell count, total protein concentration, and fibrinogen concentration in peritoneal fluid that were not statistically different from those calves that did not have abomasal puncture. Therefore, accidental puncture of the abomasums did not appear to contribute to the increase of total protein or fibrinogen concentration and total and differential white cell count in peritoneal fluid. One should remember that penetration of a healthy, nondistended segment of bowel is probably inconsequential, as the small hole undoubtedly will quickly seal.[10] In 1 study, penetration of a viscus occurred on 2 occasions.[11] In the former, the rumen was penetrated, and in the latter, the abomasum was punctured, and in neither case were effects directly attributable to the penetration detected.[11] Contrary to a previous report in horses,[10] in the current study, the calves' hematologic and peritoneal variables remained within the normal range without any treatment, indicating that the injury was minimal and was not sufficiently hazardous to alter fibrinogen concentration or total protein concentration. Because of calves' extremely thin body wall and the recent feeding, needle penetration of the abomasums can be difficult to avoid at the 1st site of collection. In this case, we recommend that the 2nd site be used when the calf had suckled recently because we did not observe any accident when using this site or when waiting until at least 1 hour to perform the peritoneal fluid collection at the 1st site.

Table 3. Hematology data from young Holstein calves.

Analyte	Day	n	Value	
			Median	Range (min, max)
RBC	1	9	7.36	(6.1, 9.8)
($\times 10^6$ cells/μL)	15	22	7.71	(4.2, 9.3)
	30	21	7.45	(4.4, 10.2)
Ht (%)	1	9	30	(24, 37)
	15	22	30	(17, 43)
	30	21	28	(21, 39)
Hb	1	9	8.50	(7.3, 11.8)
	15	22	8.70	(4.7, 12.3)
	30	21	8.50	(6, 11)
WBC (cells/μL)	1	9	12,650	(3,700, 18,650)
	15	19	8,950	(2,300, 20,900)
	30	21	8,250	(4,050, 15,200)
TP (g/dL)	1	9	7.3[a]	(5.6, 8.6)
	15	19	6.1[b]	(5.2, 7.2)
	30	21	6.0[b]	(5.1, 6.6)
Fibrinogen (g/dL)	1	9	0.40[b]	(0.2, 0.7)
	15	19	0.60[a]	(0.1, 1)
	30	21	0.70[a]	(0.2, 1.2)

n, number of samplings; RBC, red blood cells count; Ht, hematocrit; Hb, hemoglobin; WBC, white blood cell count; TP, total protein. Values for each analyte category followed by different letters are statistically different (Kruskal-Wallis for comparisons among 3 groups, $P < .05$).

The peritoneal fluid fibrinogen concentration is an ancillary diagnostic test in horses,[12] as it is indicative of vascular and inflammatory injuries.[13]

The statistically significant increase in the fibrinogen concentration observed at 30 days of age is most likely due to a physiological increase in the peritoneal fluid protein level at this age; however, it is possible that it was caused by local inflammation associated with the collection. Because the nucleated cell count, total protein concentration, and fibrinogen concentration of calves that had sustained abomasal puncture were not statistically different from those without puncture of a viscus, we believe the fibrinogen concentration in this study is more likely due to an age-related physiological increase. More studies comparing this age of calves with older cattle are necessary in an attempt to clarify this issue.

The fibrinogen concentration in calves of this study was similar to values reported for foals, ranging from 14 to 75 days of age both in peritoneal fluid (less than 0.20–0.40 g/dL) and in serum (0.20–0.80 g/dL),[14] but were much lower

Table 4. Hematology data from young Holstein calves with or without abomasal puncture.

Analyte	Abomasal Punction			
	Yes		No	
	n	Value Median (min, max)	n	Value Median (min, max)
RBC (× 10⁶ cells/μL)	11	7.72 (4.83, 9.29)	41	7.36 (4.23, 10.23)
Ht (%)	11	37 (23, 43)	41	29 (17, 39)
Hb	11	8.9 (6.8, 12.3)	41	8.5 (4.7, 11.8)
WBC (cells/μL)	9	8,150 (2,300, 14,650)	40	9,850 (3,700, 20,900)
TP (g/dL)	9	6.0 (5.6, 7.3)	40	6.1 (5.1, 8.6)
Fibrinogen (g/dL)	9	0.7 (0.2, 1.2)	40	0.6 (0.1, 1.2)

n, number of samplings; RBC, red blood cells count; Ht, hematocrit; Hb, hemoglobin; WBC, white blood cell count; TP, total protein. Values for each analyte category did not differ at 5% (Wilcoxon's rank sum test for comparisons between animals with and without abomasal punction, $P > .05$).

when compared with normal and parturient adult cows and in cattle with peritonitis.[11] This reinforces the fact that we should consider the age and species of animal when evaluating peritoneal fluid parameters.

The slightly cloudy appearance of the peritoneal fluid has been reported in a previous study in normal calves.[8] None of the samples had visual lipidic aspect that could confer an opaque aspect of the peritoneal fluid.[13]

Total protein concentrations of the peritoneal fluid were greater than those previously reported in newborn calves,[8] in parturient cows,[11] and in calves at 8 weeks of age[4]; however, they were similar to the values described in healthy adult cows.[7,11] Increased total protein concentrations can be related to the measurement technique used (refractometry × biuret) or to contamination of the sample with blood.[15] In the current study, there was a decrease in the peritoneal fluid protein concentration in calves between the 1st and the 30th day of age; the initial values could be related to increased protein associated with the transfer of maternal immunoglobulins to the neonate.

Peritoneal fluid specific gravity was greater in calves of this study than in 8-week-old calves or adults[4,7]; however, no data was found in the literature for the age range of calves in this study. Similar to other variables, such as total protein concentration and fibrinogen concentration, increased specific gravity may reflect the normal physiological status of calves until 30 days old.

Total nucleated cell counts were similar to values described by others[4,7,11] but were lower when compared with a previous report in young calves[8] and in another report in diseased adult cows.[16] Unlike in a previous report,[8] statistically significant changes in the mean neutrophil count were not observed in the calves of the current study. In the previous report, authors attempted to explain the mild increase in neutrophil count as a possible sequelae of repeated abdominal paracentesis in the calves. However, previous studies (without a negative control) with several peritoneal fluid collections by the same tap,[17] and another study,[12] with samplings by the same tap over 15 days, with a negative control (peritoneal saline injection), the total nucleated cells remained near the baseline values, which suggests these stimuli are not sufficient to evoke a high migration of polymorphonuclear cells to the abdominal cavity.[18] Serial abdominocenteses has been shown not to adversely clinically affect cattle in one study.[7] Similarly, because we did not observe any increase in the nucleated cell count, it is not likely that the 1 or 2 taps every 15 days would provoke such an alteration. Alternatively, another possible explanation was that, at the time of the last sampling, this increase could reflect a response of the peritoneal cavity to gastrointestinal tract changes associated with the introduction of dry feed prior to the final sampling.[8] However, the calves in the current study began to receive ration on the 7th day after birth, before the 2nd sampling, and this, contrary to others' concerns,[8] probably was not the primary stimulus for our findings. If so, we would have likely found differences in the neutrophil counts from the 2nd sampling onward. In our opinion, this may reflect a physiological local immune response that occurs in calves during their growing period.

The cause of the greater number of mononuclear cells in the peritoneal fluid of calves is presently unknown.[4] The predominance of mononuclear cells (mesothelial/macrophage) was also observed in calves from 2–60 days old,[4,8] whereas, in adult cattle, polymorphonuclear cells were the major constituent of the peritoneal fluid.[4,7] The mean relative and absolute macrophages/mesothelial cell values in the calves of this study were high compared with previous reports.[4,8,11] An explanation for these values could be that our calves had access to pasture to graze, whereas the animals of the other reports were housed individually in stalls. This may stimulate the immune system due to the presence of other animals or antigens found on the property (in dust, graze, water tanks, etc).

The mean relative number of lymphocytes was similar to those found for young calves[8] and adults,[4] whereas small differences were found for absolute numbers. Young calves had increased absolute values when compared with normal, parturient, or cows with peritonitis,[11] but when compared with another study,[16] these values were 4 times lower than values in adult cattle with reticuloperitonitis.

The assessment of cellularity was based on a modification of a technique that has been described in several studies.[8,19] The modification involved the inclusion of eosinophils in the polymorphonuclear cell category because the eosinophils are rarely found in the peritoneal fluid from normal cattle.[6] However, as an exception, a few samples containing a great number of eosinophils have been collected from clinically normal cattle.[6] Mesothelial cells and macrophages were grouped in the same category to decrease the possibility of errors during

the identification of these cells because, according to others,[20] it is often very difficult or even impossible to differentiate with certainty these 2 cell types in routine stained smears of peritoneal fluid. The presence of activated and/or transformed mesothelial cells also makes it difficult to distinguish the 2 cell types.

The presence of activated mesothelial cells/macrophages in the peritoneal fluid frequently is related to nonseptic inflammatory effusions and exudates; thus, their presence indicates a state of mesothelial proliferation and subsequent desquamation of cells into the peritoneal fluid.[20] The turnover of peritoneal cells can serve as a stimulus for the activation of mesothelial cells/macrophages. The technique of sampling could serve as an inflammatory stimulus leading to the activation of cells in some animals in which the sampling was repeated or difficult.[6]

The red blood cell count is highly influenced by contamination from peripheral blood during the sampling, but it has not been a frequent variable evaluated in the peritoneal fluid. Blood contamination occurred on the basis of color (mild reddish) of the peritoneal fluid and low RBC count on cytologic slides but was not considered to significantly change the interpretation of the peritoneal fluid data as previously shown.[7] Blood contamination of up to 17% of the sample volume did not significantly change the nucleated cell count, differential count, or total protein concentration of peritoneal fluid in horses.[21,22] In our study, the blood contamination was 3 times lower than that observed by others[8] at the 1st sampling (28×10^3 erythrocytes/μL versus 90×10^3 erythrocytes/μL), similar at the 2nd sampling (46×10^3 erythrocytes/μL versus 40×10^3 erythrocytes/μL), and 50% lower at the 3rd sampling (22×10^3 erythrocytes/μL versus 40×10^3 erythrocytes/μL), corresponding to 0.73, 1.96, and 0.52% of blood contamination in the peritoneal fluid for the 1st, 2nd, and 3rd samplings, respectively. Therefore, peripheral blood contamination did not appear to contribute to the increased total protein concentration in the peritoneal fluid in these animals.

This study provides reference ranges of peritoneal fluid variables for normal animals in different age ranges that can be compared with similar-aged animals with any type of gastrointestinal tract or abdominal cavity disease to be used as a possible predictive value for outcome. Additionally, accidental abomasal puncture does not alter values of fibrinogen, total protein, and nucleated cell count in peritoneal fluid and does not cause apparent clinical abnormalities.

Footnotes

[a] Compaz, Pharmacon, Itapira, SP, Brazil
[b] Dorcipec, Vallée, Montes Claros, MG, Brazil
[c] Xilocaína 2%, AstraZeneca Ltd, Cotia, SP, Brazil
[d] I-Cath, Becton-Dickinson Ltd, Juiz de Fora, MG, Brazil
[e] Vacutainer, Becton-Dickinson Ltd, Plymouth, UK
[f] Hand-held refractometer, Inlab, São Paulo, SP, Brazil
[g] SAS v.8, Statistical Analysis System, SAS Institute, Cary, NC
[h] ProcNPAR1WAY, SAS Institute, Cary, NC

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