Effect of semen in urine specimens on urine protein concentration determined by means of dipstick analysis

Laurie G. Prober, VMD; Cheri A. Johnson, DVM, MS; N. Bari Olivier, DVM, PhD; Jennifer S. Thomas, DVM, PhD

Objective—To determine the effect of semen in urine specimens on urine protein concentration measured by means of dipstick analysis.

Sample Population—14 urine samples from 3 adult castrated male dogs and 14 semen samples from 7 adult sexually intact male dogs.

Procedures—Serial dilutions of the whole ejaculate or spermatozoa-free seminal fluid in urine were created, and unaltered and diluted urine samples were analyzed by means of a commercially available dipstick; pH and specific gravity of the samples were also measured. Spermatozoa and WBC counts of the semen samples and protein concentration of the seminal fluid were determined.

Results—Protein concentrations determined by means of dipstick analysis of urine samples to which whole ejaculate (dilutions of 1:1, 1:2, 1:16, 1:64, and 1:256) or seminal fluid (dilutions of 1:1, 1:2, 1:16, and 1:64) had been added were significantly higher than concentrations in unaltered urine samples. All 13 samples to which whole ejaculate was added at a dilution of 1:2 and 10 of 12 samples to which seminal fluid was added at a dilution of 1:2 were positive for blood on dipstick analysis. There was no significant linear correlation between spermatozoa or WBC count of the semen sample and protein concentration of the spermatozoa-free seminal fluid.

Conclusions and Clinical Relevance—Results suggested that regardless of whether spermatozoa were present, semen contamination could result in false-positive results for protein and blood during dipstick analysis of urine samples from sexually intact male dogs. (*Am J Vet Res* 2010;71:288–292)

In humans, semen contamination has been shown to alter measured urine protein concentrations. In 1 study,[1] for instance, postcoital urine samples from 6 of 22 men had some degree of proteinuria, as determined by means of dipstick analysis, even though results of dipstick analysis of precoital samples from all of the men were negative for protein. In another study,[2] clinically relevant protein concentrations were found in initial-stream, but not midstream, morning urine samples from men who had had intercourse the previous evening, and in a separate study,[3] protein concentrations of postcoital urine samples in which spermatozoa were detected ranged from 100 to > 300 mg/dL, even though precoital urine samples were negative for protein. Finally, the addition of WE or spermatozoa-free SF to urine has been shown to result in proteinuria.[3]

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From the Departments of Small Animal Sciences (Prober, Johnson, Olivier) and Pathobiology and Diagnostic Investigation (Thomas), College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824. Dr. Prober's present address is Veterinary Specialists of Rochester, 825 White Spruce Blvd, Rochester, NY 14623.
Address correspondence to Dr. Prober (laurieprober@yahoo.com).

Abbreviations

SF	Seminal fluid
WE	Whole ejaculate

To our knowledge, there are no published reports of the effects of semen contamination on urine protein concentration in dogs. The purpose of the study reported here, therefore, was to determine the effect of semen in urine specimens on urine protein concentration measured by means of dipstick analysis.

Materials and Methods

Urine collection and evaluation—Fourteen urine samples from 3 adult castrated male dogs owned by members of our hospital staff were used in the study. For collection of urine samples, the prepuce and penis were cleaned with 0.05% chlorhexidine solution, and urine was collected by means of aseptic catheterization. Urine samples were tested with a commercially available dipstick[a] for glucose, bilirubin, ketones, protein, urobilinogen, and blood, and samples for which results of dipstick analysis for glucose, bilirubin, ketones, and protein were negative and results of dipstick analysis for urobilinogen were the lowest reading were submit-

ted to the clinical pathology laboratory for a complete urinalysis. Results of the dipstick analysis for protein were recorded as 0, trace, 1, 2, or 3. Results of the dipstick analysis for blood were scored as 0 (negative), 1 (trace of nonhemolyzed blood), 2 (moderate amount of nonhemolyzed blood), 3 (trace), 4 (small amount), 5 (moderate amount), or 6 (large amount).

For the complete urinalysis, 5 mL of urine was centrifuged at 485 × *g* for 5 minutes, and the supernatant was collected for determination of protein concentration and specific gravity. A single drop of unstained urine sediment was placed on a slide, and 10 hpfs (40× objective) were examined microscopically. Urine protein concentration was assessed semiquantitatively by means of a sulfosalicylic acid turbidometric assay and quantitatively by means of a microprotein assay and automated analyzer.[b] Urine pH was determined with a pH meter.[c,d] Specific gravity was determined with a refractometer. Only samples without any microscopic evidence of casts, crystals, bacteria, or spermatozoa and with < 2 WBCs/hpf and < 15 RBCs/hpf were used in the study. Urine samples were refrigerated until used; all urine samples were used within 4.5 hours after collection.

Semen collection and evaluation—Fourteen semen samples collected from 7 dogs by means of manual stimulation were also used in the study. Dogs were part of breeding colonies involved in 2 unrelated research projects and were maintained in compliance with USDA and National Institutes of Health guidelines for animal use and care. The Michigan State University Institutional Animal Care and Use Committee approved the study protocol.

For each of the semen samples, spermatozoa and WBC counts were determined with a hemocytometer. Direct smears stained with a modified Wright stain were examined, and densities of spermatozoa and RBCs were estimated on the basis of evaluation of 10 low-power fields (10× objective). An aliquot of each semen sample was saved for use as WE, and the remainder was used to obtain spermatozoa-free SF. To separate the spermatozoa from the SF, semen samples were centrifuged at 1,450 × *g* for 10 minutes, and the supernatant was immediately removed. Protein concentration of the SF was determined with an automated analyzer, and pH was determined with a pH meter. All WE and SF samples were refrigerated until used; all samples were used within 4.5 hours after collection.

Study protocol—To determine the effects of WE and SF on measured urine protein concentrations, aliquots of WE or SF (0.25 to 2.0 mL, depending on the volume of WE or SF available) were added to equal volumes of urine from the 3 castrated male dogs. These samples were then serially diluted with urine to obtain WE-to-total volume of urine and SF-to-total volume of urine ratios of 1:2, 1:16, 1:64, 1:256, 1:1,024, and 1:2,048. For each of the final samples, protein concentration was measured with a dipstick,[a] pH was determined with a pH meter, and specific gravity was determined with a refractometer.

Statistical analysis—Repeated-measures ANOVA followed by the Dunnett test was used to compare values for continuous data (ie, specific gravity and pH measured with a pH meter) between unaltered urine samples and urine samples to which WE or SF had been added. For these analyses, values of $P \leq 0.05$ were considered significant.

For ordinal data (ie, protein concentration and pH measured by means of dipstick analysis), the Wilcoxon matched pairs test was used to compare values for unaltered urine samples with values for samples to which WE or SF had been added. For each variable, 5 dilutions were examined. Therefore, to maintain an overall type 1 error rate ≤ 5%, a Bonferroni adjustment was performed, with values of $P \leq 0.01$ considered significant.

For the semen samples, simple linear correlation was used to test for a linear relationship between WBC count of the semen sample and protein concentration of the SF and between spermatozoa count of the semen sample and protein concentration of the SF.

Table 1—Results of analysis of 14 semen samples (WE) from 7 adult sexually intact male dogs.

Sample No.	No. of spermatozoa/μL	No. of WBCs/μL	Protein (mg/dL)	No. of spermatozoa/lpf	No. of RBCs/lpf	Blood score on dipstick analysis
1[a]	40,389	444	3,511	80	1.6	6
2[b]	138,000	556	2,610	111	0.2	6
3[a]	62,250	278	3,144	28	0.2	6
4[c]	53,250	2,611	ND	24	0.1	5
5[a]	45,500	556	ND	34	0	6
6[c]	19,100	189	3,034	14	0	6
7[a]	160,000	0	4,047	131	0	5
8[d]	103,000	278	2,765	57	0	5
9[e]	66,000	222	800	82	0	0
10[a]	25,800	100	2,362	35	0	6
11[e]	0	389	1,500	0	0	1
12[f]	31,500	611	1,123	25	0	5
13[g]	10,889	333	1,647	19	0	4
14[a]	190,000	56	1,971	168	0	6

lpf = Low-power field (20× objective); 10 fields were examined. ND = Not determined.

[a–g]Samples with the same superscript letter were obtained from the same dog.

Results of the dipstick analysis for blood were scored as 0 (negative), 1 (trace of nonhemolyzed blood), 2 (moderate amount of nonhemolyzed blood), 3 (trace), 4 (small amount), 5 (moderate amount), or 6 (large amount).

Results

Semen evaluation—For the 14 semen samples, spermatozoa counts ranged from 0 to 190,000/µL and WBC counts ranged from 0 to 2,611/µL (**Table 1**). Low numbers of RBCs were seen in 4 samples during examination of direct smears. Protein concentration of the spermatozoa-free SF ranged from 800 to 4,047 mg/dL (protein concentrations were not determined for 2 samples).

Effect of WE or SF on results of urine evaluation—Addition of WE or SF to urine samples resulted in detectable proteinuria (**Tables 2 and 3**). Protein concentrations determined by means of dipstick analysis in samples to which WE (dilutions of 1:1, 1:2, 1:16, 1:64, and 1:256) or SF (dilutions of 1:1, 1:2, 1:16, and 1:64) had been added were significantly ($P = 0.005$) higher than concentrations in unaltered urine samples.

Mean ± SD pH of the unaltered urine samples was 6.86 ± 0.36. Addition of WE ($P < 0.001$) or SF ($P = 0.006$) at a dilution of 1:2 was associated with a significant decrease in pH, with a mean pH decrease of 0.17 following addition of WE and a mean pH decrease of 0.27 following addition of SF. Addition of WE or SF at dilutions ≥ 1:16 did not result in any significant differences in pH.

Mean ± SD specific gravity of the unaltered urine samples was 1.023 ± 0.008 (range, 1.005 to 1.031). Addition of WE at dilutions ≥ 1:2 and addition of SF at dilutions ≥ 1:16 were not associated with any significant changes in specific gravity. Specific gravity of the samples when SF was added at a dilution of 1:2 (mean ± SD, 1.020 ± 0.005) was significantly ($P = 0.006$) lower than the specific gravity of the unaltered urine samples.

Six of the 14 unaltered urine samples were positive for blood on dipstick analysis (Tables 2 and 3). All 13 samples to which WE was added at a dilution of 1:2 and 10 of 12 samples to which SF was added at a dilution of 1:2 were positive for blood (ie, dipstick score ≥ 1). Addition of WE at a dilution of 1:2 significantly ($P = 0.002$) increased the dipstick score for blood.

Table 2—Results of dipstick analysis for protein and blood of urine samples to which WE was added at various dilutions.

					Ratio of WE to urine									
	Unaltered urine		Unaltered WE		1:2		1:16		1:64		1:256		1:1,024	
Semen sample No.	Protein	Blood	Protein	Blood	Protein	Blood	Protein	Blood	Protein	Blood	Protein	Blood	Protein	Blood
1	0	0	2	6	2	6	1	2	1	0	Tr	0	ND	ND
2	0	0	3	6	2	6	1	4	1	3	Tr	0	ND	ND
3	0	0	2	6	2	5	1	4	1	3	Tr	3	0	0
4	0	1	2	5	2	5	2	2	1	2	Tr	1	Tr	1
5	0	2	3	6	2	6	1	4	1	4	Tr	4	0	4
6	0	0	2	6	2	6	2	2	1	1	Tr	0	Tr	0
7	0	0	2	5	2	4	2	1	1	0	1	0	Tr	0
8	0	0	2	5	2	4	2	0	1	0	1	0	Tr	0
9	0	0	2	0	2	4	1	0	Tr	0	Tr	0	0	0
10	0	5	2	6	2	6	1	5	1	5	Tr	5	0	5
12	Tr*	4	2	5	2	4	1	4	Tr	3	Tr	3	0	3
13	Tr*	4	2	4	2	4	1	3	Tr	3	Tr	3	0	3
14	0	0	2	6	2	5	1	4	Tr	0	0	0	0	0

ND = Not done.
Results of the dipstick analysis for protein were recorded as 0, trace (Tr), 1, 2, or 3. Results of the dipstick analysis for blood were scored as 0 (negative), 1 (trace of nonhemolyzed blood), 2 (moderate amount of nonhemolyzed blood), 3 (trace), 4 (small amount), 5 (moderate amount), or 6 (large amount). Semen sample 11 was not tested because no spermatozoa were seen in the sample.
*Results were 0 at the onset of the study and Tr at the end.

Table 3—Results of dipstick analysis for protein and blood of urine samples to which spermatozoa-free SF was added at various dilutions.

					Ratio of SF to urine									
	Unaltered urine		Unaltered SF		1:2		1:16		1:64		1:256		1:1,024	
Semen sample No.	Protein	Blood	Protein	Blood	Protein	Blood	Protein	Blood	Protein	Blood	Protein	Blood	Protein	Blood
1	0	0	2	4	2	3	1	0	Tr	0	Tr	0	ND	ND
2	0	0	3	6	2	6	1	4	1	0	Tr	0	ND	ND
3	0	0	2	4	2	4	1	3	0	0	0	0	ND	ND
5	0	2	3	6	2	6	1	4	1	4	Tr	3	0	4
6	0	0	0	4	2	3	1	0	1	0	Tr	0	Tr	0
7	0	0	2	5	2	0	2	0	1	0	Tr	0	Tr	0
8	0	0	2	3	2	0	2	0	1	0	Tr	0	Tr	0
9	0	0	2	4	1	3	1	0	Tr	0	Tr	0	0	0
10	0	5	2	4	2	5	1	5	Tr	5	0	5	ND	ND
11	0	5	2	1	2	5	1	5	Tr	5	0	5	ND	ND
12	Tr*	4	ND	ND	2	3	1	3	1	3	0	1	0	1
13	Tr*	4	2	3	2	3	1	1	1	1	Tr	1	0	1

Semen samples 4 and 14 were not tested because of an insufficient volume.
See Table 2 for key.

Association of WBC and spermatozoa counts with SF protein concentration—There was no significant (P = 0.317) linear correlation between spermatozoa count of the semen sample and protein concentration of the spermatozoa-free SF for the 12 SF samples for which protein concentration was determined. Similarly, there was no significant (P = 0.327) linear correlation between WBC count of the semen sample and protein concentration of the spermatozoa-free SF.

Discussion

Results of the present study indicated that semen contamination could result in false-positive results for protein and blood during dipstick analysis of urine samples from dogs. Even small amounts of WE or SF were found to alter the results of dipstick analysis of urine samples.

Because alkalinity can lead to false-positive results for protein during dipstick analysis of urine samples from dogs,[4] pH was measured in the present study and the effect of addition of WE or SF on urine pH was assessed. Mean pH of unaltered urine samples was acidic, and the addition of WE or SF further decreased the pH. Thus, positive results for protein in samples to which WE or SF had been added in the present study were likely not a result of alkalinity.

Similarly, high specific gravity can lead to false-positive results for protein during dipstick analysis of urine samples from dogs. Thus, we also measured the specific gravity of samples in the present study. There were no significant changes in specific gravity with the addition of WE at dilutions ≥ 1:2 or with the addition of SF at dilutions ≥ 1:16. With the addition of SF at a dilution of 1:2, there was a significant decrease in specific gravity, but the absolute change was small and would likely not have explained the positive protein results.

In dogs, semen is ejaculated in 3 fractions: the prespermatozoa fraction, the spermatozoa-rich fraction, and the third fraction. Both the first and third fractions originate from the prostate. The protein concentration varies among fractions, with the spermatozoa-rich fraction having the highest protein concentration, followed by the third fraction.[5] Knowing that the spermatozoa-rich fraction of canine semen has the highest protein content, we separately investigated the effects on urine protein concentration of WE, which included all 3 fractions, and spermatozoa-free SF. The single semen sample that did not contain any spermatozoa was used to evaluate the effects of SF but not WE. Our study design and findings were similar to results of a study[3] of the effects of semen contamination on protein concentration in urine samples from people. In that study, addition of WE or SF at a dilution of 1:2 to urine from men resulted in protein concentrations, determined by use of dipstick analysis, > 300 mg/dL, whereas addition at dilutions of 1:40 resulted in protein concentrations of approximately 30 mg/dL and addition of WE at a dilution of 1:80 still resulted in trace proteinuria. In the present study, addition of WE at dilutions up to 1:256 or SF at dilutions up to 1:64 resulted in detectable proteinuria. For purposes of comparison, a 1:64 dilution would be equivalent to 0.078 mL of semen in 5 mL of urine.

During ejaculation, spermatozoa travel via the ductuli deferentia to the urethra, where they mix with prostatic fluid to form the ejaculate. Although most of the ejaculate exits from the penis, retrograde flow into the bladder can occur in dogs.[6] Retrograde flow can also occur at times other than ejaculation. In a study[7] of pre- and postejaculation urine samples from 6 sexually intact male dogs, for instance, spermatozoa were not only found in postejaculation samples from all 6 dogs but were also found in preejaculation urine samples from 4 of the 6 dogs. Similarly, in a separate study,[8] spermatozoa were found in urine samples from 13 of 15 sexually intact dogs that had not had any exposure to sexually intact female dogs for at least 4 weeks and had never undergone semen collection. In addition, all 15 dogs had spermatozoa in their urine after ejaculation. Finally, retrograde flow of spermatozoa into the urinary bladder can occur as a result of xylazine administration[8] and can be diminished by administration of phenylpropanolamine.[7] Results of the present study, therefore, suggest that because of possible retrograde flow of spermatozoa and SF into the urinary bladder, the potential effects of semen contamination should be considered when evaluating the results of urinalysis in sexually intact male dogs.

In the present study, spermatozoa count was not significantly correlated with protein concentration of the spermatozoa-free SF. Thus, although a finding of spermatozoa on microscopic examination of a urine sample would be indicative of semen contamination, a false-positive protein result cannot be ruled out simply because spermatozoa are not observed. Our findings for urine samples to which SF was added indicated that semen contamination of urine could cause proteinuria in sexually intact male dogs regardless of whether spermatozoa were present. Unfortunately, in the absence of spermatozoa, there is no convenient method by which to differentiate SF contamination from other causes of persistent proteinuria in sexually intact male dogs or to quantify the relative contributions of various sources. In dogs, the largest contribution to SF volume is from the prostate,[5] and it seems likely that certain prostatic disorders could contribute additional protein to the SF, just as pyuria increases the protein concentration of urine.

Finally, in the present study, the addition of WE or SF to urine resulted in positive reactions for blood during dipstick analysis, even in the absence of microscopic evidence of RBCs. Even though the dipstick score for blood was significantly increased only with the addition of WE at a dilution of 1:2, a false finding of a positive blood reaction during routine urinalysis could be of clinical concern. The reaction in the dipstick pad for blood can be catalyzed by heme groups and peroxidase activity. Human and equine SF have been shown to contain catalase peroxidase and glutathione peroxidase,[9–12] and human SF and spermatozoa have been shown to have b-type cytochrome, which contains heme.[13] Although it has not been shown whether canine SF or spermatozoa have similar components, our results indicate that in dogs, semen contamination of urine can cause false-positive reactions for blood during dipstick analysis of urine samples. Therefore, microscopic eval-

uation of the urine sediment should be performed to assess for true hematuria.

a. Bayer Multistix, Siemens Clinical Diagnostics, Elmhurst, Ill.
b. Olympus AU640e automated analyzer, Melville, NY.
c. Pinnacle 530 pH meter, Corning Inc Life Sciences, Lowell, Mass.
d. Probe (No. 476346), Corning Inc Life Sciences, Lowell, Mass.

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