Short Communication

Measurement of Alkaline Phosphatase in Canine Seminal Plasma – An Update

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Contents

In dogs, diagnosis of incomplete ejaculation and azoospermia in dogs can be done by measuring the activity of the enzyme alkaline phosphatase (AP) in seminal plasma. However, even though upper cut-off value of 5000 IU/l is given in the literature, results by different assays may vary considerably. Furthermore, no data exist concerning the stability of the enzyme during storage of frozen seminal plasma, and no recommendations for pre-analytic dilutions can be found. During the present study, we compared results from a conventional large scale wet chemistry analyzer to a widely used dry chemistry point of care system (POC) and established a best practice for pre-analytical dilutions. Furthermore, stability of enzyme activities in seminal plasma during storage at −18°C for 24 h was evaluated. The average activity of AP in the 2nd fraction of normal ejaculates measured by Reflotron® was 107 328 IU/l. After 24 h of frozen storage, activities did not differ significantly (96 544 IU/l, p > 0.05). Fresh and frozen samples were analysed in parallel by the POC and conventional chemistry analyser, and the results compared that did not reveal a significant difference (p > 0.05). A dilution of seminal plasma with physiologic saline 1 : 100 prior to analysis was sufficient for the qualitative information whether AP activity is below or above 5000 IU/l. Present data show that AP measurement by a POC dry chemistry system is sufficiently accurate in diluted seminal plasma for the diagnosis of azoospermia and that seminal plasma can be stored frozen for 24 h before analysis.

Introduction

Diagnosis of epididymis lesions causing incomplete ejaculation and azoospermia in dogs can be done by measurement of alkaline phosphatase activity (AP) in seminal plasma. AP catalyses the transport of phosphate groups into spermatozoa thereby contributing to processes important for fertilization. AP activity can be decreased after infectious, traumatic or thermal lesions of the epididymis, by autoimmune reactions or tumours (Allen and Renton 1982; Freshman et al. 1988; Olson 1991; Olson et al. 1992), as well as by obstruction or aplasia of efferent semen ducts (Althouse et al. 1993). AP activity is highest in the epididymis (Muller 1983; Frenette et al. 1986), incomplete ejaculation therefore results in AP activities ≥5000 IU/l, which can be used for diagnostic purposes (Freshman et al. 1988; Johnston 1991; Johnston et al. 2001).

Although most assays are standardized according to the International Federation of Clinical Chemistry (IFCC), results still show considerable variability. This might be due to different instrumentality and pre-analytical conditions such as sample preparation and storage. The Reflotron® (Roche Diagnostics, Vienna, Austria) is a point of care (POC) dry chemistry system widely used in veterinary practices. Thus, it seemed useful to evaluate its diagnostic utility for diagnosing incomplete ejaculation and azoospermia in dogs and to compare with a conventional large scale wet chemistry analyzer. Owing to high activities of AP in normal canine seminal plasma exceeding the linearity range, pre-analytical dilutions are necessary before analysis. Furthermore, in practice, it might not be feasible to perform analysis immediately after sample collection. However, no data are available concerning the stability of AP during frozen storage of canine seminal plasma. Further, it was investigated, whether enzyme activities in seminal plasma change significantly when stored at −18°C for 24 h.

Material and Methods

Semen was collected from 40 male dogs of different breeds, body weights (bw 19.4 ± 9.0 kg) and ages (5.1 ± 3.3 years) according to Seager and Fletcher (1972). All dogs were healthy breeding dogs. Only samples with normospermia, assessed using a Computer Assisted Sperm Analyzer (CASA, Sperm Vision®, Minitüb, Tiefenbach, Germany) as described by Schäfer-Somi et al. (2006) and Schäfer-Somi and Aurich (2007), were included. 1st, 2nd and 3rd fractions of each ejaculate were centrifuged separately (2× at 1200 g for 10 min). One aliquot was frozen stored at −18°C for 24 h, and the other was analysed immediately.

Aliquots were measured in parallel by a conventional chemistry analyzer (Hitachi 911®, Roche Diagnostics) and dry chemistry (Reflotron®; Roche Diagnostics). For the Reflotron®, test strips with a measurement range of 20–1250 IU/l, were used (Reflotron®; Alkalische Phosphatase, Ref 1 1622773; Roche Diagnostics). The 2nd fraction had to be diluted with physiologic saline until a result could be obtained, and the 1st and 3rd fractions were measured undiluted. For the auto analyzer Hitachi 911®, an IFCC standardize assay using a special dye (Alkalische Phosphatase flüssig®, ALP-IFCC flüssig, Roche #217 29 68) was used. The measurement range was 1–1200 IU/l, and the analytic sensitivity 0.67 IU/l. Dilutions were performed with physiologic saline.

Data are given as average ± standard deviation (X ± SD). Agreement between methods was tested by visual inspection of a method comparison plot where a Passing and Bablok fit has been applied for the assessment of systematic and proportional error, and
Results

The average activity of AP in the 1st, 2nd and 3rd fractions of normal ejaculates measured by Reflotron® is given in Table 1. After 24 h of storage, activities were not significantly different (p > 0.05). In both series, the values measured with Hitachi 911® did not differ significantly from the index test (n.s., Table 1). The results of both methods show acceptable agreement although a small constant bias of 80 U/l and a proportional bias of 1.75 could be detected (Fig. 1). Table 1. AP activities in fresh seminal plasma and after 24 h of frozen storage (IU/l).

The bias at a cut-off level of 3000 IU/l was 434 IU/l. There was a positive correlation between the activity of AP and the spermatozoa concentration (p < 0.01), but not with the body weight (p > 0.05).

As a best practice to spare reagents for the Reflotron®, a dilution at a ratio of 1 : 100 prior to measurement can be recommended. If the enzyme activity in the sample is considerably below 5000 and a result of <20 IU/l (lower detection limit) is obtained, no result will be given. It can be safely assumed that the AP activity is <5000 IU/l; thus, the patient is affected by the conditions under investigation. If a value of <50 IU/l is obtained, the activity is still <5000 IU/l; however, no further dilution is necessary. In case the activity is >1250 IU/l, the analyser demands further dilution that is not necessary as the qualitative information regarding AP activity to be above 5000 IU/l is sufficient for the diagnostic purpose under investigation.

Discussion

In the present study, a point of care (POC) system widely used in veterinary practices was compared to a conventional chemistry analyzer for the measurement of AP activity in canine seminal plasma. We found that the

POC system can be safely used and has some advantages: samples do not have to be transported, and the system is less prone to a bias caused by cell clots. The present study shows that the measurement of AP in canine seminal plasma by the Reflotron® is a sufficiently accurate method and that a dilution of seminal plasma 1 : 100 prior to analysis yields qualifying results for the diagnosis of incomplete ejaculation and azoospermia. Furthermore, the present data show that AP activity was stable when seminal plasma was stored frozen for 24 h. At last, we found a positive correlation between AP activity and spermatozoa concentration. This is in accordance with findings from Mollo et al. (1997) and from Girgiss et al. (1981) (humans) and Pesch et al. (2006) (horses).

In conclusion, the measurement of AP in canine seminal plasma with Reflotron® is a sufficiently accurate method under the condition that adequate partitioning of semen has been achieved during sample collection. Centrifuged seminal plasma can be stored for 24 h at −18°C before analysis. Dilution of seminal plasma of the 2nd fraction at a ratio of 1 : 100 is sufficient before analysis, and thus, reagent strips can be spared.

Table 1. AP activities in fresh seminal plasma and after 24 h of frozen storage (IU/l)

<table>
<thead>
<tr>
<th></th>
<th>Fresh X ± SD (n)</th>
<th>24 h frozen X ± SD (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hitachi® 1st</td>
<td>4288 ± 12 204³ (27)</td>
<td>3886 ± 11 245³ (24)</td>
</tr>
<tr>
<td>Reflotron® 1st</td>
<td>3300 ± 7030³ (23)</td>
<td>2656 ± 633² (18)</td>
</tr>
<tr>
<td>Hitachi® 2nd</td>
<td>87 722 ± 65 446³ (31)</td>
<td>90 644 ± 89 560³ (30)</td>
</tr>
<tr>
<td>Reflotron® 2nd</td>
<td>107 329 ± 82 326³ (28)</td>
<td>96 844 ± 75 827³ (33)</td>
</tr>
<tr>
<td>Hitachi® 3rd</td>
<td>3240 ± 11 773³ (29)</td>
<td>2798 ± 10 214³ (31)</td>
</tr>
<tr>
<td>Reflotron® 3rd</td>
<td>1717 ± 360³ (29)</td>
<td>5958 ± 23 153³ (28)</td>
</tr>
</tbody>
</table>

AP activities (IU/l) measured using Reflotron® and Hitachi® 911 using fresh and frozen seminal plasma.

1st, first fraction of the ejaculate; 2nd, second fraction; 3rd, third fraction.

a,b,cNumbers with equal indices within one row and one column do not differ significantly (p > 0.05; comparison within fractions only).

Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

The first author supervised the study and developed the draft, the second author was responsible for sampling, dry chemistry and data evaluation, and the third author was responsible for the wet chemistry measurements and statistical calculations. All authors contributed to the draft.

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References


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