The effects of short-interval ejaculation on semen quality and some biochemical parameters in dogs


SUMMARY

In the present study, ten ejaculates were obtained from each of seven German shepherd dogs. Only the sperm-rich fractions of ejaculates from each dog once a week with a mean interval of 60 min, were used during five weeks. Semen quality was examined in total of 70 semen samples. After this examination, semen samples were centrifuged (5000 g x 10 minutes) and seminal plasma were separated. Plasma total protein, calcium, phosphorus, magnesium, sodium and potassium and alkaline phosphatase were determined. The semen quality and biochemical findings were compared between the first and the second ejaculate following very-short interval ejaculation.

There were no significant differences in the percentage of motility, live spermatozoa, acrosomal, tail and total morphological defects of the second ejaculate compared with that of the first ejaculate. However, there were significantly lower values for the volume and the spermatozoal concentration in the second ejaculate than that of the first ejaculate (p < 0.05). There were significantly lower values for the total protein, calcium, magnesium and alkaline phosphatase of the second ejaculate compared with that of the first ejaculate. Positive correlations were found between spermatozoal concentration and AP ; motility and sodium concentration. The semen quality in the first and the second ejaculate in all dogs was normal for fertility. In conclusion, when the first ejaculate is inadequate, collection of second ejaculate may be useful for cryopreservation or insemination with fresh semen in dogs.

KEY-WORDS : dog - semen - ejaculation frequency - seminal plasma - biochemical parameters.

Introduction

Semen parameters such as semen volume, sperm concentration, spermatozoal morphology, motility, the numbers of live and dead spermatozoa in the second ejaculate (sperm rich) are frequently examined to assess the fertility of dogs. But these characteristics are not accurate determiners of fertility. Biochemical evaluation of seminal plasma quality is an important consideration in assessing fertility levels and for male reproductive disorders diagnosis [3, 15].

There have been few studies of the effect of ejaculatory frequency upon semen characteristics in dogs [4, 18, 20]. The only available information about the effect of very short-interval second ejaculation on the semen quality of dogs is England’s [8] investigation. ENGLAND [8] reported that there was no significant improvement in semen quality following a short-interval second ejaculation and there was no change in the quality of the ejaculated spermatozoa and that motility and morphological characteristics did not decline in the second ejaculate. ENGLAND and ALLEN [9] suggested

RÉSUMÉ


Dans la présente étude, dix ejaculats ont été obtenus de chacun de sept chiens Bergers allemands. Seules les fractions riches en sperme ont été collectées de l’animal, une heure après les premiers, une fois par semaine. La qualité des spermes a été examinée sur un total de 70 échantillons. Après cet examen, les échantillons de sperme ont été centrifugés (5000 g x 10 minutes) et le liquide séminal récupéré. Les taux de protéines totales, calcium, phosphore, magnésium, sodium, potassium et phosphatases alcalines ont été mesurés. La qualité du sperme et les paramètres biochimiques ont été comparés sur les premier et deuxième ejaculats séparés par des temps très courts.

La motilité, l’acrosome, la queue et les défauts morphologiques des spermatozoïdes, de même que la morphologie des spermatozoïdes morts dans les deuxième ejaculats ne présentent pas de différences significatives comparés à ceux des premiers ejaculats. Par contre, la concentration en spermatozoïde des seconds ejaculats est significativement plus faible que celle des premiers (p < 0.05). De même, les taux de protéines totales, de calcium, de magnésium et de phosphatases alcalines sont significativement plus bas dans les deuxième ejaculats comparés aux premiers. Des corrélations positives ont été trouvées entre la concentration en spermatozoïdes et le taux de phosphatases alcalines ; la motilité et la concentration de sodium. La qualité du sperme des premiers et deuxième ejaculats est normale du point de vue de la fertilité chez tous les chiens. Pour conclure, en cas de première ejaculation inadéquate, la collecte du second ejaculat peut être utile pour la cryopréservation ou l’insémination chez le chien.

that dogs with poor semen quality could be fertile when assisted reproductive techniques were used. CHECK and CHASE [5] reported that in oligospermic men can achieve an improved sperm concentration by performing a second ejaculation 30 to 60 minutes later.

COMHAIRE et al. [7] stated that the abnormality in the biochemical composition and physical properties of seminal plasma might indicate male infertility that will be more interesting to the research of the constituents of seminal plasma. The presence of abnormal levels of calcium and magnesium or trace elements may affect spermatogenesis with regard to production, maturation, motility and fertilizing capacity of the spermatozoa [1, 24, 26].

Alkaline phosphatase [AP] in seminal plasma is measured by the same methods as in the serum and it is typically greater than 5000 U/L [up to 40,000] in fertile dogs [14]. The major portion of this enzyme in dog seminal plasma does not come from the prostate but from epididymis [10]. A concentration of AP ≥ 5000 U/L is good evidence of the presence of the second fraction. The low concentration of AP may be evident if the second fraction is little or no second fraction collected or bilateral obstruction of the vasa deferentia or epididymides is present [11, 14]. On the other hand, if AP is high but no spermatozoa are present in the second fraction, gonadal dysfunction or bilateral blockage proximal to the tail of the epididymis is possible [19].

The aim of this study was to compare semen quality and some biochemical parameters in seminal plasma of 2 ejaculates collected following short-interval ejaculation.

Materials and methods

ANIMALS

A total of 7 German shepherd dogs were used in this study. Their ages ranged from 2 to 6 years and they were known to be fertile and healthy. The dogs had been sexually rested for at least a week before the study.

SEMEN COLLECTION AND EVALUATION

Only the sperm-rich fractions of ejaculates were collected from each dog once a week by digital manipulation in the presence of teaser bitch, with an interval of 60 minutes. Ten ejaculates were obtained during five weeks into prewarmed glass tubes from each of seven dogs for a total of 70 samples. After collection, the ejaculates were immediately placed in an incubator at 37°C, then carried to the laboratory for semen evaluation. Semen volume and sperm concentration were recorded. Sperm concentration was assessed using haemacytometer. The percentage of progressively motile spermatozoa was estimated by microscopic examination at x 400 magnifications on a prewarmed slide. The numbers of live spermatozoa and spermatozoal morphology were examined on nigrosin/eosin stained smears using the classification of CHRISTIANSEN [6]. Using this classification, individual sperm cells were recorded as being either dead (unstained) or live (stained) and their individual morphological abnormalities were tabulated according to their site (head, mid piece, and tail). The percentage of acrosomal abnormalities was evaluated after Giemsa stain.

BIOCHEMICAL ANALYSIS

After spermatological examination, a total of 70 semen samples were centrifuged (5000 g x 10 minutes) and seminal plasma were separated at room temperature and stored -20°C until biochemical analysis. Plasma total protein, calcium, phosphorus, magnesium, sodium and potassium and AP were determined by using Technicon DAX 72 autoanalyzer and its commercial kits.

STATISTICAL ANALYSIS

Statistical analysis was performed by using the Wilcoxon paired non-parametric test, and was used to evaluate comparing both spermatological and biochemical parameters in two ejaculates. Pearson Correlation Test was used to determine associations between spermatological and biochemical findings.

Results

The spermatological characteristics and biochemical parameters of the first and the second ejaculate are given in Tables I and II. The results of Pearson Correlation Tests were shown in Tables III and IV.

Spermatological findings

There were no significant differences in the percentage of motility and live spermatozoa, acrosomal and tail abnormality and total morphological defects of the second ejaculate compared with that of the first ejaculate (Table I). But there were significant lower values for the volume and the spermatozoal concentration in the second ejaculate than that of the first ejaculate (p < 0.05). There were statistically significant differences (p < 0.05) in morphologically head and middle piece between the first and the second ejaculate.

Biochemical findings

When biochemical data of the first and the second ejaculate were examined, there were significantly lower values for the total protein, magnesium and AP of the second ejaculate. On the other hand, there were no significant differences for calcium, phosphorus, sodium and potassium between the first and second ejaculate.

When studying correlations between semen and biochemical parameters, positive correlations were found between spermatozoal concentration and AP (r = 0.865, p < 0.01 in the first ejaculate ; r = 0.847, p < 0.01 in the second ejaculate) and motility and sodium concentration (r = 0.935, p < 0.01 in the first ejaculate ; r = 0.969, p < 0.01 in the second ejaculate) (Tables III and IV).

Discussion and conclusions

Although several workers have investigated the effect of ejaculation upon semen quality in dogs [4, 18, 20], the only available information about the effect of very short-interval second ejaculation on the semen quality of dogs is ENGLAND’S [8] investigation. No studies have documented
TABLE I. — Mean spermatological parameters of semen obtained from the first and the second ejaculate.

<table>
<thead>
<tr>
<th>Spermatological Parameters</th>
<th>First Ejaculate (n=35)</th>
<th>Second Ejaculate (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>3.26±0.55</td>
<td>2.42±0.39*</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>84.42±5.3</td>
<td>82.42±4.7</td>
</tr>
<tr>
<td>Concentration (x10^9)</td>
<td>398.28±60.4</td>
<td>333.71±60.6*</td>
</tr>
<tr>
<td>Live spermatozoa (%)</td>
<td>75.48±1.95</td>
<td>74.96±1.96</td>
</tr>
<tr>
<td>Head abnormality (%)</td>
<td>2.26±1.18</td>
<td>2.85±0.91*</td>
</tr>
<tr>
<td>Middle piece abnormality (%)</td>
<td>0.99±0.60</td>
<td>1.44±0.63*</td>
</tr>
<tr>
<td>Tail abnormality (%)</td>
<td>2.94±1.45</td>
<td>2.78±1.23</td>
</tr>
<tr>
<td>Acrosome abnormality (%)</td>
<td>1.71±1.25</td>
<td>1.62±0.76</td>
</tr>
<tr>
<td>Total morphological defect (%)</td>
<td>7.92±23</td>
<td>8.66±1.79</td>
</tr>
</tbody>
</table>

* Values of p < 0.05 were considered to be statistically significant in columns. Data expressed as mean ± SD

TABLE II. — Mean biochemical parameters of seminal plasma for the first and the second ejaculate.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>First Ejaculate (n=35)</th>
<th>Second Ejaculate (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein (g/l)</td>
<td>39.1±7.7</td>
<td>28.5±4.8*</td>
</tr>
<tr>
<td>Calcium (mEq/l)</td>
<td>0.87±0.06</td>
<td>0.92±0.13</td>
</tr>
<tr>
<td>Phosphorus (mEq/l)</td>
<td>0.96±0.23</td>
<td>0.83±0.18</td>
</tr>
<tr>
<td>Magnesium (mEq/l)</td>
<td>1.85±0.31</td>
<td>1.57±0.21*</td>
</tr>
<tr>
<td>Sodium (mEq/l)</td>
<td>142.52±6.2</td>
<td>141.23±7.9</td>
</tr>
<tr>
<td>Potassium (mEq/l)</td>
<td>12.90±1.78</td>
<td>12.29±1.11</td>
</tr>
<tr>
<td>AP (U/l)</td>
<td>4189±526.1</td>
<td>3521±414.0*</td>
</tr>
</tbody>
</table>

* Values of p < 0.05 were considered to be statistically significant in columns. Data expressed as mean ± SD

In conclusion, the present study indicates that when the first ejaculate is inadequate, collection of the second ejaculate may be useful to increase the number of spermatozoa. We, therefore, conclude that short-interval ejaculations do affect the density, volume, and change the some biochemical parameters of the semen. Generally the potential fertility of the dogs, which have normal initial sperm characteristics, was not changed. In the future, research on semen quality and biochemical constituents of seminal plasma of infertile dogs should also be investigated.
References