Assessment of semen quality in infertile dogs using computer-assisted sperm analysis by the Hamilton-Thorne Semen Analyser

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Abstract

Semen quality parameters of infertile and fertile dogs were compared. Sperm concentration and semen motility parameters were measured by the Hamilton-Thorne Semen Analyser IVOS 12.3. The spermatozoal morphology and the percentage of live spermatozoa were examined microscopically. Forty-six dogs of various breeds were examined. Twenty dogs had a conception failure within last year. These dogs had a history of prior normal fertility. Twenty six fertile dogs served as control. All animals underwent a clinical examination as well as ultrasonography. Sperm concentration was significantly lower in infertile dogs than in fertile dogs. For most determined motility parameters (MOT, PMOT, VAP, VSL, VCL, BCF, RAPID, STATIC) significant differences between infertile and fertile dogs were found. The percentage of spermatozoa with normal morphology also significantly differed between both groups. Ultrasonography of the prostate gland and testes revealed no pathological conditions. The testicular degeneration was assumed to be a possible cause of infertility in these dogs. The present study showed that the most sperm CASA motility parameters were significantly lower in infertile dogs in comparison to the fertile ones, and confirmed the usefulness of the Hamilton-Thorne Semen Analyser for a quick and objective analysis of sperm concentration and motility in dogs.

Key words: dogs, semen quality, infertility, computer analysis.

Introduction

Not much is known about infertility in male dogs. It is a complex phenomenon classified as congenital or acquired infertility. The acquired infertility can result from several problems including poor semen quality (3, 5, 8). The diagnosis of infertility involves history, general and genital clinical examination, libido evaluation, semen collection, and assessment and complementary examinations, such as hormonal evaluation, serological or bacteriological tests, testicular biopsy, etc. Till now evaluation of semen quality (i.e. sperm concentration, motility, and morphology) was based on subjective microscopic techniques (8, 13, 19). However, the variability of results obtained with the use of traditional methods of semen assessment is high (7, 18). Computer-assisted sperm analysis (CASA) system allows an accurate and objective assessment of different semen parameters such as concentration, total and progressive motility, and different velocity parameters (21, 26). The technical settings for dog semen were standardised, and reference values for fertile dogs were provided (9, 20, 26). CASA systems were successfully used for comparing different sperm extenders for chilling and cryopreservation of dog sperm (7, 15, 23, 25, 26), and for assessing the effect of various additives on motility of frozen-thawed dog semen (12, 23). However, there are only few studies on the relationship between sperm characteristics assessed by CASA and fertility in dogs. Rijsselaere et al. (20), and Niżyński et al. (16) have found significant differences between fertile and subfertile dogs in most of the sperm parameters assessed by CASA. Thus, the aim of the study was to
evaluate semen quality parameters in infertile dogs by
the Hamilton-Thorne Semen Analyser.

Material and Methods

The study was carried out on 46 dogs of various
breeds. Twenty dogs displayed conception failure
within at least 3 (3-5) matings of fertile bitches during
last year. These dogs had a history of prior normal
fertility. Twenty six fertile dogs from different kennels
served as a control group. The age of dogs ranged from
4 to 8 years. All males were in good general condition.
Prostate gland and testes were examined by ultrasound.
Sperm was collected by manual manipulation as
described by Linde-Forsberg (10) in the presence of a
teaser bitch in heat. The ejaculate was collected into a
prewarmed (36-38°C) glass tube.

For the assessment of sperm morphology, Diff-
Quick stain was used. The percentage of live and dead
spermatozoa was estimated on dried smears with
eosin/nigrosin. Two hundred spermatozoa were
evaluated per slide representing 100%.

The sperm concentration and motility parameters
were evaluated by Hamilton Thorne Sperm Analyser,
version IVOS 12.3 (HTR-IVOS 12.3). This CASA
system consists of a phase-contrast microscope,
camera, minitherm heating stage, image digitizer, and
computer saving and analysing the data. The software
settings are shown in the Table 1.

Table 1. Software settings of the HTR IVOS 12.3 used
in the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamber type</td>
<td>Leja 4</td>
</tr>
<tr>
<td>Temperature of analysis (°C)</td>
<td>38.0</td>
</tr>
<tr>
<td>Fields acquired</td>
<td>10</td>
</tr>
<tr>
<td>Frame rate (Hz)</td>
<td>60</td>
</tr>
<tr>
<td>Minimum contrast</td>
<td>75</td>
</tr>
<tr>
<td>Minimum cell size (pixels)</td>
<td>6</td>
</tr>
<tr>
<td>Straightness (STR)</td>
<td>75</td>
</tr>
<tr>
<td>Vap cut-off (µm/s)</td>
<td>9.9</td>
</tr>
<tr>
<td>Prog.min VAP (µm/s)</td>
<td>100.0</td>
</tr>
<tr>
<td>VSL cut-off (µm/s)</td>
<td>20.0</td>
</tr>
</tbody>
</table>

After semen collection, the sperm concentration of
the 2nd fraction was first estimated using a microscope.
The normal ejaculates were diluted with physiological
saline/Tris basis at a (vol/vol) ratio 1:3 to about
50 × 10⁶/mL directly before the analysis. In the cases of
oligospermia, semen was not diluted. There were no
cases of azoospermia in these dogs. Subsequently, 2 µL
of diluted semen was mounted in a disposable Leja
counting chamber and was allowed to settle on the
minitherm heating stage (38°C) before the analysis.
The following parameters were measured:

counting chamber and was allowed to settle on the
diluted semen was mounted in a di

concentration (CONC), percentage of motile
spermatozoa (MOT), percentage of spermatozoa with a
progressive motility (PMOT), velocity average
pathway (VAP), velocity straight line (VSL), velocity
curvilinear (VCL), amplitude lateral head (ALH), beat
cross frequency (BCF), straightness (STR), and
linearity (LIN). According to the low VAP cut-off and
medium VAP cut-off, the sperm population was
additionally divided into four categories: RAPID,
MEDIUM, SLOW, and STATIC.

Statistical analysis. The results were presented as
mean values with standard deviation indicated (mean
± SD). The sperm quality parameters were compared
between both groups using a Mann-Whitney or
Student’s t-test (GraphPAD PRISM, Version 4.00,
GraphPad Software, USA). The level of significance
was set at P < 0.05.

Results

All dogs were clinically healthy and showed
normal libido. Ultrasonography of the prostate and
testes revealed no pathological conditions. The mean
values of semen quality parameters of fertile and
infertile dogs are shown in Table 2.

The sperm concentration was significantly (P < 0.05)
lower in infertile dogs than in fertile dogs (40.15 ±
36.56 × 10⁶/mL vs 205.32 ± 132.1 × 10⁶/mL). For most
motility parameters determined (MOT, PMOT, VAP,
VSL, VCL, BCF, RAPID, STATIC), significant
differences (P < 0.05) between infertile and fertile dogs
were found. The percentage of spermatozoa with
normal morphology also differed significantly
(P < 0.05) between both groups (66.4 ± 19.47% vs
82.08 ± 6.33%).

Discussion

In the study, the sperm quality parameters of
fertile and infertile dogs obtained by the Hamilton-
Thorne Semen Analyser IVOS 12.3 were compared. In
the infertile dogs, sperm concentration, percentage of
spermatozoa with normal morphology, and the majority
of most of the evaluated motility parameters were
significantly (P < 0.05) lower than in fertile dogs. The
results were in agreement with those reported by
Rijselaere et al. (20) and Niażński et al. (16). They
have found, that almost all of the evaluated sperm
characteristics was significantly poorer in dogs with
lower fertility.

Generally, outcomes from routine laboratory sperm
assays are poorly correlated with male fertility (17, 22).
Oettle (18) reported that the fertility in dogs was
adversely affected, when the percentage of normal
morphology was <60%. The motility parameters are
essential for the fertilisation process in other species (1, 6).
In female dogs, the pregnancy rates were significantly influenced by the total number of progressively motile sperm cells per ejaculate (13).

The most useful method of discrimination between semen of fertile and infertile dogs seems to be the evaluation of velocity parameters (VAP, VSL, VCL) and BCF. These parameters are probably important for the progression of spermatozoa into cervical mucus and the penetration of zona pel lucida of oocytes (26). Fertilisation rates of human oocytes in vitro have been shown to correlate positively with sperm velocity (1, 14). Computer sperm analysis is an accurate technique used for the estimation of motility parameters of the same ejaculates (14). Computer sperm analysis is an accurate technique used for the assessment of the motility parameters of canine semen. High number of spermatozoa can be analysed individually in a short period (7, 9, 20, 26).

The assessment of canine sperm quality parameters by Hamilton-Thorne semen analyser, complementary to the clinical examination, allows the elimination of clear-cut cases of infertility in the male dogs. A common cause of acquired infertility in the male dogs are prostatic and testicular diseases (3, 5, 8).

In the study, all infertile dogs were clinically healthy and the cause of poor semen quality could not be diagnosed. According to Johnston et al. (8), the reason of male infertility remains unknown in 70% of cases. The testicular degeneration was assumed as the possible cause of the determined oligo- and asthenozoospermia in these dogs which is a common phenomenon. A variety of causative factors, such as hormonal disturbances, heat, stress, toxins, and autoimmune disorders have been reported (3).

The study showed that most of the determined sperm characteristics were significantly lower in infertile dogs in comparison to the fertile males, and confirmed the usefulness of the Hamilton-Thorne semen analyser for a quick and objective analysis of sperm concentration and motility in dogs. After correct calibration, the measurements are easy to perform and can be done by technicians under the veterinary surgeon’s control. CASA system proved its usefulness in routine veterinary practice, which provides hope for more common use of this equipment in the future.

Table 2. Sperm quality parameters (mean ± SD) in infertile (n = 20) and fertile (n = 26) dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Infertile dogs (n = 20)</th>
<th>Fertile dogs (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of the ejaculate</td>
<td>mL</td>
<td>2.71 ± 1.11</td>
<td>2.98 ± 0.96</td>
</tr>
<tr>
<td>(sperm-rich fraction)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>x 10^{9}/mL</td>
<td>40.15 ± 36.56^{a}</td>
<td>205.32 ± 132.16^{b}</td>
</tr>
<tr>
<td>MOT</td>
<td>%</td>
<td>62.6 ± 30.91^{c}</td>
<td>85.31 ± 8.8^{c}</td>
</tr>
<tr>
<td>PMOT</td>
<td>µm/s</td>
<td>33 ± 23.61^{d}</td>
<td>58.84 ± 15.33^{d}</td>
</tr>
<tr>
<td>VAP</td>
<td>µm/s</td>
<td>109.34 ± 45.03^{e}</td>
<td>146.65 ± 10.89^{e}</td>
</tr>
<tr>
<td>VSL</td>
<td>µm/s</td>
<td>97.90 ± 39.08^{e}</td>
<td>127.32 ± 15.54^{e}</td>
</tr>
<tr>
<td>VCL</td>
<td>µm/s</td>
<td>158.46 ± 60.59^{e}</td>
<td>209.95 ± 17.32^{e}</td>
</tr>
<tr>
<td>ALH</td>
<td>µm</td>
<td>3.42 ± 2.02</td>
<td>5.67 ± 0.78</td>
</tr>
<tr>
<td>BCF</td>
<td>Hz</td>
<td>12.85 ± 9.83^{f}</td>
<td>22.91 ± 3.66^{f}</td>
</tr>
<tr>
<td>STR</td>
<td>%</td>
<td>85.15 ± 20.63</td>
<td>86.61 ± 4.36</td>
</tr>
<tr>
<td>LIN</td>
<td>%</td>
<td>61.95 ± 20.96</td>
<td>64.76 ± 9.06</td>
</tr>
<tr>
<td>RAPID</td>
<td>%</td>
<td>38.55 ± 26.64^{g}</td>
<td>63.92 ± 17.51^{g}</td>
</tr>
<tr>
<td>MEDIUM</td>
<td>%</td>
<td>24.2 ± 18.83</td>
<td>19.5 ± 9.11</td>
</tr>
<tr>
<td>SLOW</td>
<td>%</td>
<td>13.9 ± 14.72</td>
<td>11.42 ± 8.45</td>
</tr>
<tr>
<td>STATIC</td>
<td>%</td>
<td>23.50 ± 29.05^{g}</td>
<td>4.88 ± 4.23^{g}</td>
</tr>
<tr>
<td>NORMAL</td>
<td>%</td>
<td>66.4 ± 19.47^{c}</td>
<td>82.08 ± 6.33^{c}</td>
</tr>
<tr>
<td>LIVE (eosin/nigrosin)</td>
<td>%</td>
<td>85.10 ± 15.23</td>
<td>89.23 ± 4.41</td>
</tr>
</tbody>
</table>

^{a,b} Values with different superscripts are statistically different at P < 0.05

References
2. Farrel P.B., Presicce G.A., Brockett C.C., Foote R.H.: Quantification of bull sperm characteristics measured by