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

Anna Domosławska, Sławomir Zduńczyk, Wojciech Niżański, Tomasz Janowski

Department of Animal Reproduction with Clinic, Faculty of Veterinary Medicine, University of Warmia and Mazury, 10-719 Olsztyn, Poland
1Department of Reproduction and Clinic for Farm Animals, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, 50-366 Wrocław, Poland

anna.domoslawska@gmail.com

Received: May 27, 2013 Accepted: September 13, 2013

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 Niżański 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: 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 Niżański 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 pellucida of oocytes (26). Fertilisation rates of human oocytes in vitro have been shown to correlate positively with sperm velocity (1, 24). In bovine, sperm velocity is highly correlated with the 59 d non-return rate (2). In humans, VCL and BCF were significantly higher for spermatozoa which penetrated in sperm penetration assay than for those, which failed to penetrate (4). Thus, not only assessment of the percentage of motile spermatozoa is important, but also velocity parameters are different depending on the fertility of the male.

The assessment of sperm motility using the conventional microscopical methods is difficult and subjective. High variations have been reported 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.

### References

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

---

### 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 (sperm-rich fraction)</td>
<td>mL</td>
<td>2.71 ± 1.11</td>
<td>2.98 ± 0.96</td>
</tr>
<tr>
<td>Concentration</td>
<td>x 10^8/mL</td>
<td>40.15 ± 36.56^a</td>
<td>205.32 ± 132.16^a</td>
</tr>
<tr>
<td>MOT</td>
<td>%</td>
<td>62.6 ± 30.91^b</td>
<td>85.31 ± 8.8^b</td>
</tr>
<tr>
<td>PMOT</td>
<td>µm/s</td>
<td>33 ± 23.61^a</td>
<td>58.84 ± 15.33^a</td>
</tr>
<tr>
<td>VAP</td>
<td>µm/s</td>
<td>109.34 ± 45.03^a</td>
<td>146.65 ± 10.89^a</td>
</tr>
<tr>
<td>VSL</td>
<td>µm/s</td>
<td>97.90 ± 39.08^a</td>
<td>127.32 ± 15.54^a</td>
</tr>
<tr>
<td>VCL</td>
<td>µm/s</td>
<td>158.46 ± 60.59^a</td>
<td>209.95 ± 17.32^a</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^a</td>
<td>22.91 ± 3.66^a</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^a</td>
<td>63.92 ± 17.51^a</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^*</td>
<td>4.88 ± 4.23^*</td>
</tr>
<tr>
<td>NORMAL</td>
<td>%</td>
<td>66.4 ± 19.47^b</td>
<td>82.08 ± 6.33 ^b</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>

* Values with different superscripts are statistically different at P < 0.05
computer-assisted sperm analysis (CASA) and their relationship
3. Feldmann J., Nelson R.W.: Canine and feline endocrinology and
718–733.
4. Fetterlof P.M., Rogers B.J.: Prediction of human sperm
penetrating ability using computerized motion parameters. Mol
6. Holt C., Holt W.V., Moore H.D., Reed H.C., Curnock R.M.:
Objectively measured boar sperm motility parameters correlate
with the outcomes of on-farm inseminations: results of two
computer-based automated system for dog semen analysis.
8. Johnston S.D., Root–Kustritz M.V., Olson P.N.S.: Canine and
feline theriogenology. Edited by W.B. Saunders, Philadelphia
9. Klimowicz M., Nižanski W., Savic M., Zbyryt I., Dubiel A.:
Evaluation of dog semen quality using a conventional
microscopic method, flow cytometry and HTM IVOS computer
and chilled extended semen. Vet Clin North Am Small Anim
11. Mickelsen W.D., Memon M.A., Anderson B.P., Freeman D.A.:
The relationship of semen quality to pregnancy rate and litter
size following artificial insemination in the bitch. Theriogenology
1993, 39, 553–560.
12. Milani C., Fontbonne A., Sellem E., Steletta C., Gerard O.,
Romagnoli S.: Effect of post–thaw dilution with caffeine,
pentoxifylline, 2′–deoxyadenosine and prostatic fluid on motility
of frozen post–thawed dog semen. Theriogenology 2010, 74,
153–164.
The relationship of semen quality to pregnancy rate and litter
size following artificial insemination in the bitch. Theriogenology
1993, 39, 553–560.
control of sperm concentration and sperm motility counts in
15. Nižanski W., Klimowicz M., Partka A., Savic M., Dubiel A.:
Effects of inclusion of Equex STM into Tris–based extender on
the motility of dog spermatozoa incubated at 5 degrees C.
Mikołajewska N., Blasiak K., Mila H., Stańczyk E.: Flow
cytometric, computer assisted and traditional sperm analysis in
fertile and subfertile dogs. Proc. 14th EVSSAR Congress, Milano
2011, p. 52.
stainings and flow cytometry for canine semen assessment.
Reprod Dom Anim 2012, 47 (Suppl. 6), 215–221.
19. Rijsselaere T., Van Soom A., Tanghe S., Coryn M., Maes D., de
Kruif A.: New techniques for the assessment of canine semen
20. Rijsselaere T., Maes D., Hoflack G., de Kruif A., Van Soom A.:
Effect of body weight, age and breeding history on canine sperm
quality parameters measured by the Hamilton–Thorne analyser.
assisted sperm analysis in dogs and cats: An update after 20
years. Reprod Dom Anim 2012, 47 (Suppl. 6), 204–207.
22. Rodríguez–Martínez H.: Can we increase the estimate value of
semen assessment? Reprod Dom Anim 2006, 41 (Suppl. 2),
2–10.
23. Rota A., Milani C., Cabianca G., Martini M.: Comparison
between glycerol and ethylene glycol for dog semen
Sperm velocity and morphology, female characteristics, and the
hypo–osmotic swelling test as predictors of fertilization
potential: experience from the IVF model. Int J Fertil Womens
25. Schafer S., Aurich Ch.: Use of new computer–assisted sperm
analyzer for the assessment of motility and viability of dog
spermatozoa and evaluation of four different semen extenders for
semen analyzers in andrology research and veterinary practice.