Can semen quality be a predictor of health in working dogs?

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Introduction

• The role of working dogs in Israel security.

• Direct relationship between semen and health:
  
  Humans- mandatory semen analysis for combat pilots (In Israel)

  Animals – mandatory semen analysis for fresh use and for shipment purposes

• In working dogs with suboptimal semen parameters – some ill a few weeks later
Questions asked

• Can semen analysis be a part of the routine health exam in the working dog?
• Can routine semen analyses be used as a sole diagnostic test?
• Are other semen analysis tests necessary?
Some background

• The relationship between fertility and health is complex (Eisenberg et al, 2014)

• Environmental, hereditary and individual factors (Łepecka-Klusek C. et al 2011)

• Environment conditions affects spermatogenesis (Zou Z. et al,2011; PŠrn T. et al,2015)

• Pollution of air, water, earth, food, drinks, drugs and items in everyday use (Eisenberge et al,2015; Joffe M.,2003; Kumar S.,2004;Schmid TE et al, 2007;Sheiner EK, 2003; Stefankiewicz J et al, 2006; Wdowiak A et al, 2007)
spermatogenesis

- spermatogonium
- Primary spermatocyte
- spermatid
- spermatozoa
Oxidative Stress

- Varicocele
- Drugs
- Smoking
- Male accessory gland infections
  - Prostate glands
  - Seminal vesicle
  - Epididymis
- Immature/Abnormal Spermatozoa
- Prolonged stasis of spermatozoa in the epididymis or in transit

- H$_2$O$_2$
- O$_2$
- OH

- Protein Damage
- Lipid Peroxidation
- Biomembrane Damage
- DNA Damage

- Sperm Damage
- Infertility
Sperm production in humans is influenced by various factors, including:

- Health and occupation
- Socioeconomic status
- Age
- Exertion
- Noise
- Heat
- Vibration
- Smoking
- Shift work
The example of obesity:

- Males with excess body weight due to a surplus of adipose tissue: (Tsao C et al PLoS ONE 10(3) 2015):
  - reduced sperm numbers
  - low binding capacity
  - low fertilization ability
  - difficulties to undergo capacitation and acrosome reaction
The protectors: Molecular chaperones

- Large family of structurally diverse proteins
- Expressed in all cell types
- Chaperones – cellular resistance to environmental stressors
- Heat Shock Proteins (HSPs)
- HSP 70 family- best studied
Chaperone expression during spermatogenesis and maturation
Aberrant expression HSPs

- Arrested spermatogenesis
- Pronounced defects in sperm function
In dogs

• Semen quality and health status – scarce data

• Association between semen quality and canine health - data lacking

• Seminal HSPs and association with health - not been investigated
Study objectives

1. Determine/Study the use of semen analysis combined with reproductive ultrasonogram as a reliable method to predict working dog health status.

2. Determine whether HSP’s are present in the semen, its frequency/level/quantity and its role/association the semen quality and dogs health status.
Method- dogs:

- 36 male Malinois MECHELER 😊/Dutch shepherd/crosses
Dog groups:

1. Working dogs Group - 15 dogs (1-4 years old), that are currently under a training regime for at least one year.

2. Rested dogs Group - 10 dogs (1-4 years old), that have not worked for at least 90 days; this group will serve as a control.

3. Sick dogs Group - 11 dogs that have experienced illness, suffer from chronic illness, or are ill at the time of examination – of no more than 3 months.
Study design

• Health history was collected
• All dogs underwent:
  ❖ general physical examination
  ❖ Testicular palpation
General health parameters:

- Complete blood count
- Blood biochemical analyses
- Urine analysis
Testicular & Prostatic ultrasonography:
Semen collection

- Semen collected in three fractions:
  
  $1^{st}$ = presperm fraction
  
  $2^{nd}$ = sperm rich fraction
  
  $3^{rd}$ = prostatic fraction
Semen analysis

- Sperm count
- Motility and progressive motility
- Morphology
- Alkaline phosphatase and PH levels
- Cytology

Samples collected from all fractions for future HSPs level analyses.
Normal characteristics of the different fractions of the dog ejaculate:

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; Fraction</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; Fraction</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>0.1-2 mL (average 0.33 mL)</td>
<td>0.1-3 mL (average 1.17 mL)</td>
<td>1-2 to &gt;20 mL</td>
</tr>
<tr>
<td>Colour</td>
<td>clear or opaque</td>
<td>greyish-white, white, milky-white</td>
<td>clear, transparent</td>
</tr>
<tr>
<td>Consistency</td>
<td>watery</td>
<td>watery-milky, milky</td>
<td>watery</td>
</tr>
<tr>
<td>Character</td>
<td>prostate secretion with admixture of epithelial cells, urine, bacteria and sperm cells</td>
<td>sperm cells suspended in seminal plasma</td>
<td>prostate gland secretion</td>
</tr>
<tr>
<td>pH (average)</td>
<td>6.37</td>
<td>6.10</td>
<td>7.20</td>
</tr>
<tr>
<td>Duration</td>
<td>5-90 sec. (average 13.5 sec)</td>
<td>5-300 sec. (average 52.4 sec.)</td>
<td>60 sec-20 min. (average 6 min. 55 sec.)</td>
</tr>
</tbody>
</table>
Results

Working dogs in BLUE
Rested dogs in GREEN
Sick dogs in RED
## Vital parameters

<table>
<thead>
<tr>
<th>parameter</th>
<th>Working dogs</th>
<th>Rested dogs</th>
<th>Sick dogs</th>
<th>significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>3.9133</td>
<td>2.8736</td>
<td>5</td>
<td>Yes</td>
</tr>
<tr>
<td>BCS</td>
<td>3.018</td>
<td>3</td>
<td>2.8</td>
<td>No</td>
</tr>
<tr>
<td>Rectal temperature</td>
<td>38.777</td>
<td>38.873</td>
<td>38.867</td>
<td>No</td>
</tr>
<tr>
<td>Pulse rate</td>
<td>91.692</td>
<td>98.545</td>
<td>107.111</td>
<td>no</td>
</tr>
</tbody>
</table>
## Semen parameters

<table>
<thead>
<tr>
<th>parameter</th>
<th>Working dogs</th>
<th>Rested dogs</th>
<th>Sick dogs</th>
<th>significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction 1 Volume</td>
<td>2.25</td>
<td>2.36</td>
<td>1.62</td>
<td>no</td>
</tr>
<tr>
<td>Fraction 2 Volume</td>
<td>1.2</td>
<td>1.25</td>
<td>1.05</td>
<td>no</td>
</tr>
<tr>
<td>Fraction 3 PH</td>
<td>7.333</td>
<td>7.636</td>
<td>7.76</td>
<td>no</td>
</tr>
<tr>
<td>Total Semen conc.</td>
<td>233.82</td>
<td>311.045</td>
<td>369.75</td>
<td>no</td>
</tr>
<tr>
<td>Neuts 3rd fraction</td>
<td>0.733</td>
<td>0</td>
<td>0.2</td>
<td>no</td>
</tr>
<tr>
<td>motility</td>
<td>69.643</td>
<td>79</td>
<td>69</td>
<td>no</td>
</tr>
<tr>
<td>% normal sperm</td>
<td>63</td>
<td>72.455</td>
<td>56</td>
<td>no</td>
</tr>
<tr>
<td>ALP 2nd fraction</td>
<td>22349</td>
<td>58161</td>
<td>57530</td>
<td>no</td>
</tr>
<tr>
<td>% midpiece defects</td>
<td>15.933</td>
<td>10.909</td>
<td>22.2</td>
<td>no</td>
</tr>
</tbody>
</table>
## Semen parameters

<table>
<thead>
<tr>
<th>parameter</th>
<th>Working dogs</th>
<th>Rested dogs</th>
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<th>significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>% tail defects</td>
<td>7.733</td>
<td>3.545</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Neuts 2(^{nd}) fraction</td>
<td>0.857</td>
<td>0.5</td>
<td>1.4</td>
<td>yes</td>
</tr>
<tr>
<td>Head defects</td>
<td>13.33</td>
<td>4</td>
<td>5.6</td>
<td>tendency</td>
</tr>
<tr>
<td>Bacti 2(^{nd}) fraction</td>
<td>0</td>
<td>0.455</td>
<td>0.1</td>
<td>tendency</td>
</tr>
</tbody>
</table>

![Graph of % head defects](image1)

![Graph of bacteria 2\(^{nd}\) fraction](image2)

![Graph of neutrophils](image3)
<table>
<thead>
<tr>
<th>parameter</th>
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<th>Rested dogs</th>
<th>Sick dogs</th>
<th>significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine PH</td>
<td>7.071</td>
<td>7.313</td>
<td>7.056</td>
<td>no</td>
</tr>
<tr>
<td>Urine WBC</td>
<td>13.143</td>
<td>9.375</td>
<td>20.444</td>
<td></td>
</tr>
<tr>
<td>Urine Proteins</td>
<td>16.07</td>
<td>3.13</td>
<td>27.78</td>
<td>tendency</td>
</tr>
<tr>
<td>Urine glucose</td>
<td>0.07</td>
<td>0</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>Urine nitrites</td>
<td>0.14</td>
<td>0</td>
<td>0.11</td>
<td>no</td>
</tr>
<tr>
<td>Urine ketones</td>
<td>2.14</td>
<td>1.25</td>
<td>3.33</td>
<td>no</td>
</tr>
<tr>
<td>Urine epithelial cells</td>
<td>0.571</td>
<td>0.313</td>
<td>0.111</td>
<td>no</td>
</tr>
<tr>
<td>Urine sperm</td>
<td>1.29</td>
<td>1.50</td>
<td>1.11</td>
<td>no</td>
</tr>
<tr>
<td>Urine RBC</td>
<td>13.192</td>
<td>5.438</td>
<td>30.611</td>
<td>tendency</td>
</tr>
</tbody>
</table>
Graphs – relevant urine parameters

- Urine ery:
  - Working: Low values
  - Rested: Moderate values
  - Sick: High values

- Urine protein:
  - Working: Low values
  - Rested: Moderate values
  - Sick: High values
## Complete blood count parameters:

<table>
<thead>
<tr>
<th>parameter</th>
<th>Working dogs</th>
<th>Rested dogs</th>
<th>Sick dogs</th>
<th>significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>9.2771</td>
<td>8.7364</td>
<td>10.624</td>
<td>no</td>
</tr>
<tr>
<td>RBC</td>
<td>6.98</td>
<td>7.55</td>
<td>6.91</td>
<td>no</td>
</tr>
<tr>
<td>HGB</td>
<td>17.2</td>
<td>19.46</td>
<td>16.38</td>
<td>yes</td>
</tr>
<tr>
<td>MCHC</td>
<td>37.6</td>
<td>39.9</td>
<td>35.9</td>
<td>yes</td>
</tr>
<tr>
<td>RDW</td>
<td>12.814</td>
<td>12.027</td>
<td>13.34</td>
<td>yes</td>
</tr>
<tr>
<td>platelets</td>
<td>192.93</td>
<td>189</td>
<td>273.6</td>
<td>no</td>
</tr>
<tr>
<td>%neutrophils</td>
<td>48.257</td>
<td>45.209</td>
<td>37.11</td>
<td>no</td>
</tr>
<tr>
<td>% lymph</td>
<td>38</td>
<td>38.655</td>
<td>46.48</td>
<td>no</td>
</tr>
<tr>
<td>PCV</td>
<td>46.5</td>
<td>49.18</td>
<td>46.10</td>
<td>no</td>
</tr>
</tbody>
</table>
Graphs – blood parameters

- **HGB (Hematocrit)**
  - Normal range: 8.1-14.2 g/dL
  - Groups: working, rested, sick

- **RDW (Red Distribution Width)**
  - Groups: working, rested, sick

- **MCHC (Mean Corpuscular Hemoglobin Concentration)**
  - Groups: working, rested, sick
## Blood biochemistry

<table>
<thead>
<tr>
<th>parameter</th>
<th>Working dogs</th>
<th>Rested dogs</th>
<th>Sick dogs</th>
<th>significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>3.9</td>
<td>3.92</td>
<td>3.7</td>
<td>no</td>
</tr>
<tr>
<td>ALP</td>
<td>43.27</td>
<td>39.27</td>
<td>46.3</td>
<td>no</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.078</td>
<td>0.92</td>
<td>0.648</td>
<td>no</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>185.38</td>
<td>174.373</td>
<td>187.44</td>
<td>no</td>
</tr>
<tr>
<td>Glucose</td>
<td>96.14</td>
<td>92.7</td>
<td>92.04</td>
<td>no</td>
</tr>
<tr>
<td>Phosphate</td>
<td>3.7947</td>
<td>3.6655</td>
<td>3.888</td>
<td>no</td>
</tr>
<tr>
<td>Trigl</td>
<td>33.553</td>
<td>31.591</td>
<td>40.01</td>
<td>no</td>
</tr>
<tr>
<td>Urea</td>
<td>53.567</td>
<td>49.091</td>
<td>3.230</td>
<td>no</td>
</tr>
<tr>
<td>Na</td>
<td>44.867</td>
<td>145</td>
<td>146.2</td>
<td>no</td>
</tr>
</tbody>
</table>
Prostate lobe diameter size

- The Size of the right and left prostate of Group 2 was significantly lower ($P \leq 0.0014$) than groups 1 and 3.
So- to conclude:

• Several parameters show differences
• Few semen parameters show association or tendency for differences
• HSPs results in processing- awaiting results
• This data may be used in the future for standardization of a tool for health status evaluation in dogs
Future research

• Expand to larger numbers

• Use in different breeds

• Use in different types of working dogs

• Histones, cytokines, interleukines in seminal fluid
acknowledgements

• Dr. Yaron Bruchim
• Dr. Tal Raz
• Dr. Gila Kahila- Bar Gal
• Noy Sivan
• Dr. Shiri Novak

• The IDF Veterinarians and Soldiers without whom this research would not be conducted
Questions?