SEMEN MICROBIOTA OF PATIENTS WITH ASTENOZOOSPERMIA AND HEALTHY CONTROLS: CLUSTER ANALYSIS OF REAL-TIME PCR DATA

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Male Contribution to Infertility Worldwide

- 15% of married couples have infertility;
- Total — about 50 mln couples;
- Male infertility — 50% of all cases:
  - 20-30% "pure" male infertility;
  - 20-30% mixed male and female infertility.

Change in Sperm Count

• From 1973 to 2011 sperm count declined by 50–60% in the Western countries (North America, Europe, Australia, New Zealand);
• This trend was not characteristic of other countries.

Relevance of Studying Semen Microbiota

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<th>Male infertility remains unexplained in more than 50% of the cases</th>
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<td>Up to 15% cases of infertility — genital tract infections</td>
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<td>Less than 2% of the microorganisms can be cultured in a lab</td>
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Assessment of Semen Microbiota

- 16S rRNA gene specific Next generation sequencing
- Qualitative PCR
- Quantitative PCR (real-time PCR) – «ANDROFLOR®» Real-time PCR KIT (DNA-Technology, Russia)
<table>
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<th>Sexually transmitted pathogens (qualitative analysis)</th>
<th>C.trachomatis, N.gonorrhoeae, T.vaginalis, M.genitalium</th>
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</table>
| Gram-positive facultative anaerobes (quantitative analysis) | •Staphylococcus spp.  
•Streptococcus spp.  
•Corynebacterium spp. |
| Obligate anaerobes (quantitative analysis) | •Gardnerella vaginalis  
•Atopobium cluster  
•Megasphaera /Veilonella/Dialister  
•Sneathia/Leptotrichia/Fusobacterium  
•Bacteroides/Porphyromonas/Prevotella  
•Anaerococcus  
•Peptostreptococcus/Parvimonas  
•Eubacterium |
| **Genital mycoplasma (quantitative analysis)**          | • *Ureaplasma urealyticum*  
|                                                      | • *Ureaplasma parvum*  
|                                                      | • *Mycoplasma hominis*  |
| **Gram-negative facultative anaerobes (quantitative analysis)** | • *Haemophilus spp.*  
|                                                      | • *Pseudomonas aeruginosa/Ralstonia/Burkholderia*  |
| **Enterobacteriaceae / Enterococcus spp. group**         | • *Enterobacteriaceae spp./Enterococcus spp.*  |
| **Yeast-like fungi (quantitative analysis)**              | • *Candida spp.*  |
| **Transient microbiota (quantitative analysis)**         | *Lactobacillus spp.*  |
Study Design

• 301 patients, who came to the “Garmonia” Medical Center (Yekaterinburg, Russia) either seeking preconception care or for infertility treatment.

• Depending on the spermiogram results, they were divided into two groups.
  – Group 1 (n=171) — asthenozoospermia,
  – Group 2 (n=130) — normospermia
Methods: Semen Microbiota Evaluation

• DNA extraction
  – PREP-NA PLUS extraction kit (DNA-Technology, Russia)

• Real-time PCR
  – Androflor® REAL-TIME PCR Detection Kit (DNA-Technology, Russia)
Methods: Cluster Analysis

- Cluster analysis was performed for the samples with (total=301: asthenozoospermia=171, normospermia = 130) the total bacterial load (TBL) of at least $10^3$ GE/ml.
- Cluster analysis was conducted using the k-means++ algorithm, scikit-learn.
- The Silhouette index and the Davies–Bouldin index (DBI) were used to confirm the stability of clusters.
Example of Lab Report after Testing the Semen Microbiota Using Real-time PCR Kit «ANDROFLOR®»

- Total bacterial load: 4.2 log GE/ml
- 10 groups of microorganisms detected in quantity > 3.0 log GE/ml
4 Stable Microbiota Clusters were Distinguished

- Cluster 1 - with predominance of obligate anaerobes (OA).
- Cluster 2 - with predominance of gram-positive facultative anaerobes (GPFA)
- Cluster 3 – with predominance of *Lactobacillus spp.* (LB)
- Cluster 4 – with predominance of *Enterobacteriaceae/Enterococcus* (EE)
Cluster 1 – with Predominance of Obligate Anaerobes

Normospermia

Asthenozoospermia
Cluster 2 - with Predominance of Gram-positive Facultative Anaerobes

Normospermia

Asthenozoospermia
Cluster with Predominance of *Lactobacillus* spp.

**Normospermia**

**Asthenozoospermia**
Cluster with Predominance of Enterobacteriaceae/Enterococcus (EE)

**Normospermia**

**Asthenozoospermia**
Cluster Detection Rate: Normospermia and Asthenozoospermia

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<tr>
<th></th>
<th>Normospermia</th>
<th>Asthenozoospermia</th>
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<tbody>
<tr>
<td>OA</td>
<td>40,2%</td>
<td>50,5%</td>
</tr>
<tr>
<td>GPFA</td>
<td>25,2%</td>
<td>12,6%</td>
</tr>
<tr>
<td>LB</td>
<td>20,6%</td>
<td>25,3%</td>
</tr>
<tr>
<td>EE</td>
<td>14,0%</td>
<td>11,6%</td>
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Conclusion: Semen Microbiota in Patients with Normospermia

- Cluster formed by obligate anaerobes was not dominated by any bacteria group.
- Corynaebacterium spp., Streptococcus spp. are prevalent in the cluster formed by gram-positive facultative anaerobes.
- Lactobacilli prevalence is associated with the detection of obligate anaerobes and facultative anaerobes.
- Enterobacteriaceae spp./Enterococcus spp. group is associated with gram-positive anaerobes and obligate anaerobes, as well as low TBL.
Conclusion: Semen Microbiota in Patients with Asthenozoospermia

- One of the bacteria groups was prevalent in the obligate anaerobes cluster.
- Lactobacilli were present in the cluster where *Enterobacteriaceae spp./Enterococcus spp.* group was predominant.
- *Corynaebacterium spp., Streptococcus spp.* are prevalent in the cluster formed by gram-positive facultative anaerobes.
- Lactobacilli cluster was formed without other bacteria groups.