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P-097 The Microbiota Difference in Normal and Abnormal Semen Samples Assessed by Real-time RCR

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Study question: Does microbiota differ in semen samples with normal and abnormal parameters?

Summary answer: The higher prevalence of obligate anaerobes and lactobacilli was more typical for semen samples with abnormal parameters compared to samples with normozoospermia.

What is known already: For a long time, semen in healthy men was considered to be sterile, and the detection of microorganisms was associated with pathologies. More recently, it has been shown that microbiota can be present in semen samples with normal semen parameters. Some researchers, usually using 16S RNA sequencing, cautiously suggested that certain microbial groups could be associated with norm and pathology.

Study design, size, duration: 825 semen samples from men who came to the “Garmonia” Medical Center (Yekaterinburg, Russia) seeking pre-conception care in 2019-2021, were included in the study. Depending on the spermograms results, the samples were divided into two groups: Group 1 – normozoospermia (n = 350), Group 2 – samples with abnormal parameters (n = 475).

Participants/materials, setting, methods: Semen microbiota was analyzed by real-time PCR Androflor kit (DNA Technology, Russia).

Detection rates of 18 bacteria/bacterial groups and 4 consolidated groups of microorganisms (obligate anaerobes (OA), gram-positive facultative anaerobes (GPFA), gram-negative facultative anaerobes GNFA), and mycoplasmas) were compared in Group 1 and Group 2. Two-tailed Fisher’s exact test was performed to check the significance of the differences.

Main results and the role of chance: Bacterial DNA was detected more often in the samples with abnormal spermograms (Group 2) than in normozoospermia samples (Group 1): 79.6% and 73.4% respectively (p = 0.045). There was no difference in detection rates of GPFA, GNFA and mycoplasmas in both groups, whereas the detection rate of OA was significantly higher in Group 2 samples than in Group 1 (44.0% and 36.3%, respectively, p = 0.026).

When analyzing the detection rate of different bacteria/bacterial groups we registered significant differences for 7 of them. For all the groups, the

higher detection rate was associated with Group 2 (abnormal semen samples). In these samples, the detection rate of lactobacilli was 23.4% (compared to 13.7% in Group 1, p < 0.001), *Megasphaera spp./Veillonella spp./Dialister spp.* – 21.1 (12.0% in Group 1, p = 0.001), *Bacteroides spp./Porphyromonas spp./Prevotella spp.* – 26.7% (17.1% in Group 1, p = 0.001), *Anaerococcus spp.* – 15.2% (9.7% in Group 1, p = 0.021), *Peptostreptococcus spp./Parvimonas spp.* – 18.1% (12.3% in Group 1, p = 0.026), *Eubacterium spp.* – 24.8% (18.9% in Group 1, p = 0.043).

Limitations, reasons for caution: Androflor PCR kit uses the 10³ genome-equivalent/ml threshold for most of the target microbial groups; samples with lower DNA level are considered as negative. Hence, it is possible that the results may be different when using another threshold.

Wider implications of the findings: Further research should determine the microbiota variants in semen samples with different abnormalities, such as asthenozoospermia, leukospermia, etc. These findings could expand our understanding of the contribution made by specific microorganisms to diseases, which potentially could lead to the development of new therapeutic options or improvement of the existing ones.

Trial registration number: Ethics Committee of Ural State Medical University (Protocol № 7 dated September 20, 2019)