

**Summary answer:** The obligate anaerobes prevalence in semen microbiota was associated with reduced embryo quality in patients with asthenozoospermia, but no difference was found for normozoospermic samples.

**What is known already:** The success of the ART depends on a number of factors, including embryo quality. The viability of IVF embryo could be affected by various environmental factors, including contamination with semen microbiota. Semen is not sterile, and even processed it may contain some bacteria, which may colonize culture dishes with oocytes and embryos. It was shown that the presence of bacteria might alter the sperm quality and the contamination of embryo culture dishes with bacteria was associated with poor quality of the developing embryos.

**Study design, size, duration:** 126 infertile couples attending the “Garmonia” Medical Center (Yekaterinburg, Russia) for IVF in 2020-2021, were included in the study. Depending on the spermogram results, they were divided into two groups Group 1 (n=51) — asthenozoospermia, Group 2 (n=75) — normospermia.

**Participants/materials, setting, methods:** Semen microbiota was analyzed using RT-PCR kit Androflor (DNA-Technology, Russia). Cluster analysis was performed for 78 samples with the total bacterial load (TBL) of at least  $10^3$  GE/ml (asthenozoospermia = 31, normozoospermia = 47). Cluster analysis was conducted using the k-means++ algorithm, scikit-learn. The blastocyst development rate (BDR), as the proportion of 2PN zygotes, was determined on day 5 after injection. BDR  $\geq$  40% was considered satisfactory, BDR < 40% - unsatisfactory.

**Main results and the role of chance:** When analyzing semen microbiota, 8 stable clusters were distinguished. Cluster 1 was lactobacilli-dominated, Cluster 2 and 3 - Gram-positive facultative anaerobes dominated with prevalence of *Corynebacterium spp.* and *Streptococcus spp.* respectively; Cluster 4 – *Enterobacteriaceae/Enterococcus spp.*-dominated; Cluster 5, 6 - obligate anaerobes dominated with prevalence of *Bacteroides spp./Porphyromonas spp./Prevotella spp.* or *Peptostreptococcus spp./Parvimonas spp.*, respectively. Cluster 7 was formed by mixed obligate anaerobes (MOA); Cluster 8 - by *Mycoplasmae*.

There was no difference in detection rate of individual clusters in samples with normospermia and asthenozoospermia.

Depending on their BDR each group were divided into subgroups: Subgroup 1A (n=25) with BDR  $\geq$  40%, Subgroup 1B (n=26) with BDR < 40%; Subgroup 2A (n=38) with BDR  $\geq$  40%, Subgroup 2B (n=37) with BDR < 40%.

The detection rate of different microbiota clusters in Subgroups 2A and 2B (normospermia samples) was almost similar.

In asthenozoospermia samples the lower embryo quality (Subgroup 1B) was associated with higher prevalence of Cluster 7 (53.8%) comparing to those of Subgroup 1A (16.0%),  $p < 0.001$ . Cluster 1 was detected more often in Subgroup 1A (20.0%) than in Subgroup 1B (7.8%), but  $p > 0.05$ . TBL  $> 10^3$  GE/ml was present in 52% of Subgroup 1A samples and in 69.2% of Subgroup 1B samples.

**Limitations, reasons for caution:** Cluster analysis was not conducted for the samples with TBL lower than  $10^3$  GE/ml, since their results were incompatible with the data received for the negative control samples.

**Wider implications of the findings:** Further research could determine the significance of the described bacterial clusters in semen with other pathologies. Establishing the relationship between the characteristics of semen microbiota and poor embryo quality might allow the development of new algorithms for treating patients with reproductive disorders, depending on the composition of semen microbiota.

**Trial registration number:** not applicable

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P-104 Impact of Semen Microbiota on the Embryo Quality

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**Study question:** Is there a relationship between the microbiota composition of semen samples used for assisted reproductive technologies (ART) and quality of embryos?