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LITTLE- foetal life: sterile or not sterile?

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Background: Womb and foetus have been considered sterile until birth or rupture of the amniotic sac. Nonetheless, recent evidence suggests that placenta and amniotic fluid host a unique microbial ecology. For this reason, clarifying if the womb is colonised and at what gestational age is fundamental for the field. Due to the vast medical and scientific consequences of a “colonised womb”, it is crucial to exclude potential environmental or procedural contaminations. Here, we present preliminary results conducted on a rodent animal model.

Material/methods: Pregnant CD albino female rats were housed individually. Every two days, stool samples were collected until sacrifice (at day 16). Amniotic fluid, placentae and foetal intestines were collected in sterile conditions. Total bacterial DNA was extracted from collected samples and analysed by Next Generation sequencing techniques (NGS). Colonizing microbes were visualised *in situ*, using labelled probes targeting 16S rDNA by fluorescent *in situ* hybridization (FISH).

Results: The NGS results showed the presence of microorganisms in all examined tissues. A total of 4,670,364 raw 16S rRNA sequences were obtained; after fragment re-building and quality filtering, a total of 3,442,383 reads was counted, with a mean of 191,244 reads per sample. Firmicutes (57%) and Bacteroidetes (25%) were the most abundant phyla in all tissues. Microbial communities showed a foetus-dependent clusterisation, confirming the high inter-individual variability of commensal microbiota. The FISH analysis confirmed the microbes' existence *in situ*.

Conclusions: These preliminary results provide information about the abundance and the localisation of the microorganisms in utero. The presence of microorganisms in placentae, amniotic fluid and foetal intestines suggest an antenatal foetal microbial colonisation. These data will be confirmed in human samples.

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