

Semen metagenomics and spent culture media in patients undergoing conventional in vitro fertilisation: abridged secondary publication

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KEY MESSAGES

1. Vertical transmission of microbes was identified in 82.5% of spent embryo culture medium samples; semen was the primary source of contamination.
2. Increased abundances of *Staphylococcus* and *Streptococcus anginosus* were associated with reduced sperm count and total motility, respectively.
3. Microbiomes in spent embryo culture medium and seminal fluid were not associated with assisted reproductive technology treatment outcomes including fertilisation rates, embryo development, number of available embryos,

clinical pregnancy rates, miscarriage rates, and live birth rates.

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Introduction

In conventional in vitro fertilisation (IVF), contamination of embryo culture occurs in 0.38% to 8% of cases. The true prevalence of microbial colonisation is probably underestimated primarily due to the lack of evidence; most cases are only identified when turbidity or bacterial growth is observed. The source of contamination in culture media is usually unknown, but microbes are believed to predominantly originate from seminal fluids and are vertically transmitted to culture media during fertilisation. Bacteria have also been detected in follicular fluids. However, the association between embryo culture media contamination and assisted reproductive technology (ART) outcomes remains unclear. We hypothesised that spent embryo culture media (SECM) is not strictly sterile and that microbes may be vertically transmitted from seminal and follicular fluids into culture media during ART treatment. This study aimed to determine the prevalence of vertical microbial transmission into embryo culture media and the associations between ART outcomes and the microbiomes of SECM, semen, and follicular fluid.

Methods

Infertility patients seeking ART treatment at the Prince of Wales Hospital, Hong Kong, were invited to participate. Follicular fluid from the largest follicle was aspirated from each ovary, transferred into a sterile test tube, and centrifuged to remove debris.

Semen samples were prepared for insemination using standard density gradient centrifugation; the supernatant seminal fluid was stored at -80°C . Fertilised embryos were transferred into a new culture dish containing fresh drops (25 mL) of embryo culture media for further culture. SECM samples were stored at -80°C for microbiome analysis. Unused embryo culture medium drops served as negative controls. All samples were subjected to DNA extraction, and standardised samples were prepared for sequencing. Bioinformatics and statistical analyses were conducted.

Results and discussion

Of 196 couples recruited, 88 were excluded and 108 were included in the analysis. The mean ages of male and female participants were 36.0 and 34.8 years, with mean body mass indices of 23.8 and 22.2 kg/m², respectively (Table). The 16S rRNA amplicon analysis generated a median of 43 800 reads per sample. After decontamination, 2189 unique amplicon sequence variants were identified: 457 (21.9%) were exclusively detected in SECM, 464 (26.2%) were exclusively detected in semen, and 627 (31.5%) were exclusively detected in follicular fluids; 97 taxa (4.4%) were shared among all specimen types.

Vertical transmission of microbes was identified in 82.5% of SECM samples; semen was the primary source of contamination. Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria were the four dominant phyla (Fig). At the genus level, *Lactobacillus*, *Streptococcus*, *Staphylococcus*,

TABLE . Characteristics of participants (n=108 pairs)

Characteristic	Value*
Age, y	
Women	34.8±3.1
Men	36.0 (36.3-38.4)
Body mass index, kg/m ²	
Women	22.2 (22.3-23.8)
Men	23.8 (23.2-25.3)
Cigarette smokers	
Women (n=61)	5 (8.2)
Men (n=61)	14 (23.0)
Alcohol consumers	
Women (n=61)	7 (11.5)
Men (n=61)	10 (16.4)
Aetiology of infertility (n=61)	
Tubal occlusion	19 (31.2)
Pelvic adhesions	20 (32.8)
Endometriosis	17 (27.9)
Uterine factor	5 (8.2)
Male factor	52 (85.2)
Ovulatory disorders (including polycystic ovarian syndrome)	9 (14.8)
Coital dysfunction	3 (4.9)
Idiopathic infertility	3 (4.9)

* Data are presented as mean ± standard deviation, median (95% confidence interval), or No. (%) of participants

Bacillus, *Prevotella*, and other microbes were detected in SECM samples.

In seminal fluid, microbial taxa detected in normozoospermic men were comparable to those in men with defective semen parameters. However, increased abundances of *Staphylococcus* and *Streptococcus anginosus* were associated with reduced sperm count and total motility, respectively (P<0.001). In follicular fluid, the relative abundance of *Porphyromonas* was associated with anovulation (P<0.001); *Neisseria* was more abundant in women with uterine factor infertility than in women with other causes of infertility (P<0.01). The relative abundance of *Facklamia* was associated with unexplained infertility (P<0.01). Despite these findings, the microbiomes of SECM, seminal fluid, and follicular fluid were not associated with ART outcomes including fertilisation rates, embryo development, number of available embryos, and clinical pregnancy rates.

Conclusion

Embryo culture media is primarily contaminated by semen and, to a lesser extent, by follicular fluid. Strong associations were observed between specific microbial taxa in semen and sperm quality, as well as between follicular fluid microbiomes and the aetiology of infertility in women. However, the microbiomes of SECM, semen, and follicular fluid were not associated with ART treatment outcomes.

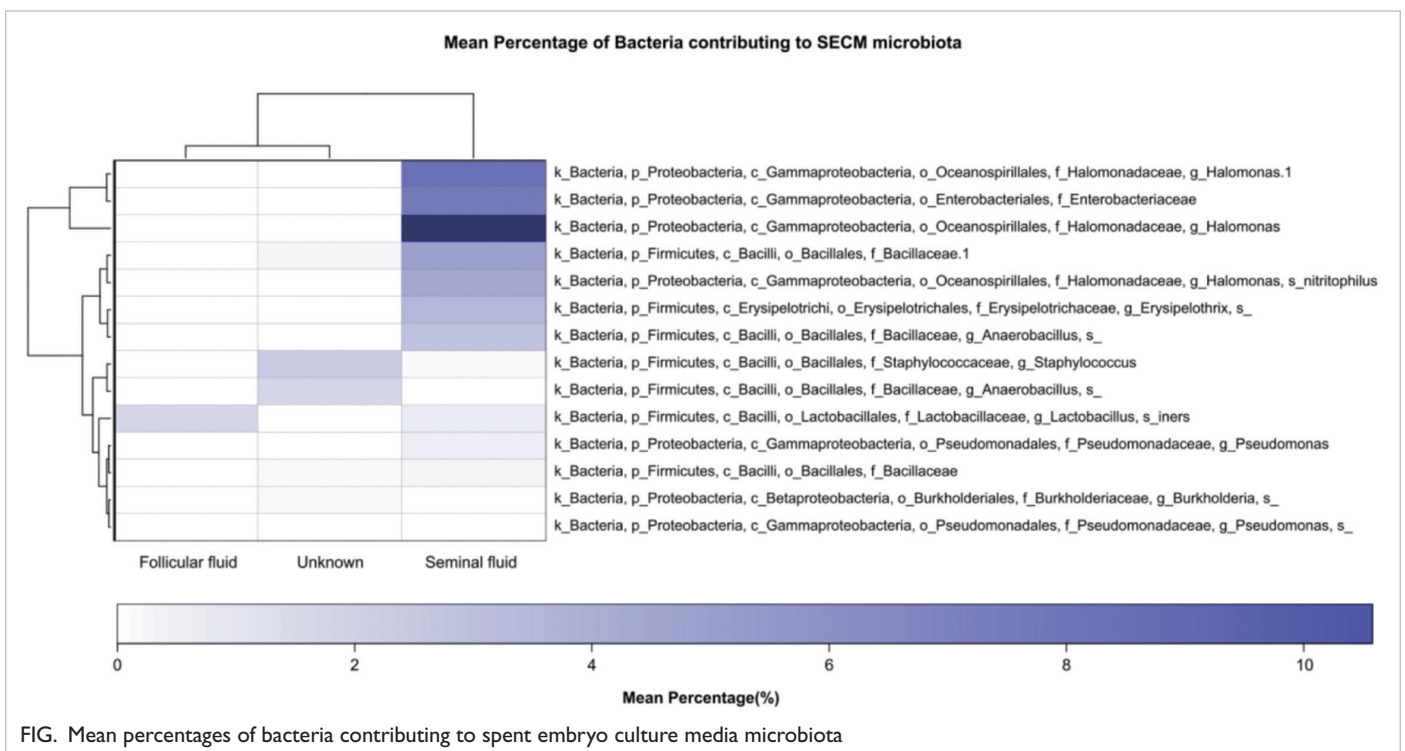


FIG. Mean percentages of bacteria contributing to spent embryo culture media microbiota

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Disclosure

The results of this research have been previously

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1. Alqawasmeh O, Fok E, Yim H, Li T, Chung J, Chan D. The microbiome and male infertility: looking into the past to move forward. *Hum Fertil (Camb)* 2023;26:450-62.
2. Alqawasmeh OAM, Jiang XT, Cong L, et al. Vertical transmission of microbiomes into embryo culture media and its association with assisted reproductive outcomes. *Reprod BioMed Online* 2024;49:103977.