Precise quantification of cell-free DNA in spent blastocyst media using a novel digital PCR technique

D. Bakalova[1], J. A. Castellón[2], I. Ceschin[2], I. M. Merino[2], M. S. Benitez[2], C. Rubio[2], L. Navarro-Sánchez[2]

[1]Igenomix UK (Part of Vitrolife Group), Guildford, United Kingdom
[2]Igenomix Spain (Part of Vitrolife Group), Valencia, Spain

BACKGROUND

During growth and development in the IVF laboratory, embryos release cell-free DNA (cfDNA) into the surrounding culture media - spent blastocyst media (SBM); this offers a non-invasive method for embryo genetic testing. Existing quantification methods struggle to precisely detect and measure the low cell-free DNA concentrations in SBM.

OBJECTIVES

1. To quantify cell-free DNA in SBM samples from human embryos.

2. To assess possible associations with day of sampling, sequencing informativity, and embryo ploidy status.

METHODS

We analysed SBM samples from 71 embryos that were cultured until day 5, 6, or 7. Quantification was performed before whole genome amplification (WGA) using resDNASEQ Human Residual DNA Quantitation System and QuantStudio Absolute-Q digital PCR instrument (ThermoFisher). A subset of 52 samples underwent next generation sequencing (NGS; Ion ReproSeq PGS kit, ThermoFisher) to ascertain the embryo's chromosomal status.

RESULTS

Figure 1

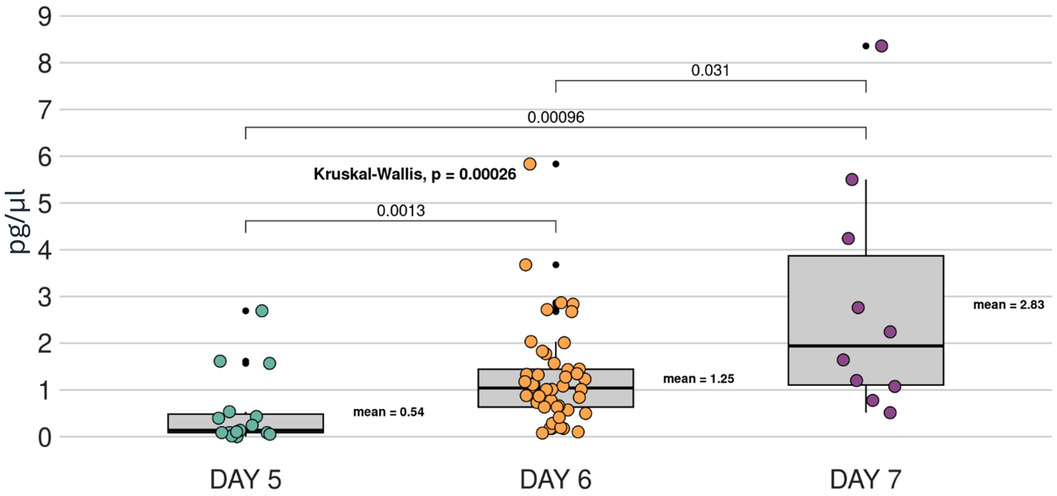


Figure 1. Cell-free DNA concentration vs. day of SBM collection.
Cell-free DNA levels were **significantly higher** in samples collected on **days 7 and 6** compared to day 5.
Day 5: 15 samples; Day 6: 46 samples; Day 7: 10 samples;

- Assuming an average SBM volume of 7µl, the estimated mean amount of cell-free DNA per sample were: 19.81pg, 8.75pg, and 3.78pg, respectively for day 7, 6, 5 samples.
- Cell-free DNA content was negligible (0.04 pg/µl) in across 20 negative controls.

Figure 2

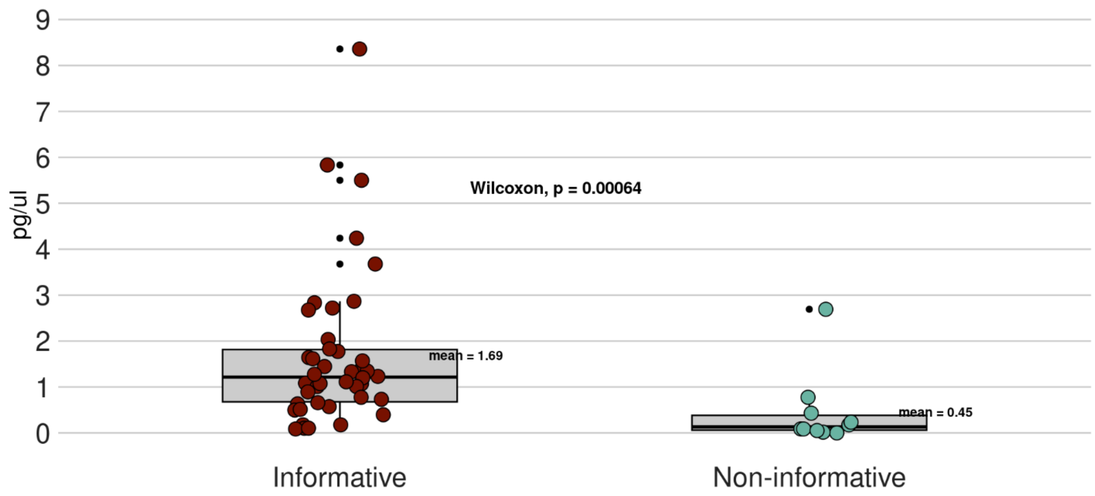


Figure 2. Cell-free DNA concentration vs. sequencing result.
- **NGS-informative** samples contained **significantly more cell-free DNA** versus non-informative samples.
Informative: 42 samples; Non-informative: 10 samples

- Assuming an average SBM volume of 7µl, the estimated mean amount of cell-free DNA per sample were: 11.83pg and 3.15pg, respectively for informative and non-informative samples.

CONCLUSIONS

This digital PCR method employed in our study is a novel technology enabling precise quantification of very low DNA quantities prior to WGA, providing an accurate insight into the cell-free DNA content in SBM. Cell-free DNA quantity peaks on days 6 and 7, indicating optimal sampling timing.