00214 In-situ Single Oocyte Quantification of DNA and mRNA Using Digital PCR Without Extraction

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Aims: Current artificial reproductive technologies (ART) such as in vitro fertilization (IVF) are relatively inefficient with only a quarter of the cycles resulting in live births. One of the challenges lies in choosing high quality oocytes based on their morphometrics, a standard practice carried out by the embryologists, but is non-quantitative and prone to bias. Thus, our study aims to develop an in-situ protocol to detect, identify and quantitatively determine genetic markers from single oocytes, destructively and non-invasively. The developed technique to pick up individual oocyte transcript differences will go towards understanding the underlying mechanisms governing high quality eggs, and their non-invasive assessment, which may eventually be employed to increase the success of IVF.

Methodology: In our study, the Clarity digital polymerase chain reaction (dPCR) platform was used. The detection limits, sensitivity and reproducibility of detection of the platform was determined using mouse lung DNA. Subsequently, mRNA and DNA from single mouse oocytes were quantified.

Result: Our results show that the Clarity dPCR system has a limit of detection of 0.1 ng/mL (r2=0.999), similar to conventional PCR. By quantifying known plasmid concentrations, we were able to robustly show linearity of quantified genetic material (r2=0.998). The duplex detection of DNA using probes is comparable to singlet detection using primers, increasing the efficiency of detection. Importantly, with the system allowing for in-situ detection without the need for purification, specific DNA and mRNA from single oocytes were easily detected directly from our samples.

Conclusion: We have shown that the Clarity dPCR system is a suitable platform to detect minute genomic markers from single oocytes and their culture supernatant to access oocyte quality, and understand transcript changes. This would pave the way for the possibility of non-invasive detection of genomic markers for accurately qualifying oocytes and embryos for ART in the near future.