been indicated as a major cause of this problem. Since maternal spindle transfer (MST) allows replacement of the entire cytoplasm of an affected oocyte, it holds promise for the enhancement of embryonic development. Recent studies in the mouse and in human oocytes donated for research have confirmed the technical feasibility of MST and provided reassurance concerning safety. Here we present results from the first registered pilot trial aiming to reveal whether MST has potential to overcome infertility caused by poor oocyte quality.

**DESIGN:** This pilot trial (ISRCTN11455145) was restricted to 25 patients, selected based on their multiple previous failed IVF attempts, in each case associated with massive embryo development arrest. Female age over 40y/o and severe male factor were exclusion criteria. Procedures were authorized by the National Authority of Assisted Reproduction (437/23.9.2016) and approved by the Hospital’s IRB. Informed consent was obtained from patients and donors to conduct all procedures and follow-up the children born.

**MATERIALS AND METHODS:** The meiotic spindle from patient’s oocytes was isolated in a minimal cytoplasmic volume and transferred to a previously enucleated donor oocyte. MST oocytes were inseminated by ECSI and cultured up to 6 days in a time-lapse incubator. Good morphology blastocysts underwent biopsy, aneuploidy testing and analysis of mitochondrial DNA (mtDNA) carrier overy levels. SNPs analysis and DNA fingerprints were used to confirm the origin of the nuclear genome and mtDNA in biopsied samples, amniotic fluid and somatic tissues of resulting children.

**RESULTS:** A total of 25 MST cycles were performed in patients with an average age of 37.1 y/o and a mean number of previous failed IVF attempts of 5.7 (min 3 and max 11). The mean number of MI oocytes used for MST per patient was 4.4. MST was applied successfully in 113 of 123 oocytes (91.9%) used. Normal fertilization was confirmed in 76.1% (86/113) of injected oocytes and 52 of these developed into good quality blastocysts (60.5%). Genetic screening revealed 50% (26/52) of embryo biopsy specimens to be euploid and mtDNA carrier overy levels <1%. In 16/25 cases, at least one euploid blastocyst of good morphology was obtained. Thus far, single blastocyst transfers were performed in 9 patients, resulting in 6 clinical pregnancies (66.7%). Two patients have delivered a healthy child and 3 more pregnancies are ongoing. Genetic analyses of the biopsied cells, amniotic fluid and samples collected after birth (blood, urine, saliva, cord blood, placenta) confirmed the parentage of the children and the origin of the donated mtDNA. Follow-up studies are being performed on the children born.

**CONCLUSIONS:** Given the difficult reproductive history of the patients, results are encouraging. However, more carefully controlled pilot trials and follow-up studies are needed to provide more insights into the efficacy of MST to overcome infertility.

**O-174 3:05 PM Monday, October 19, 2020**

**ENHANCING CELL INJECTION SYSTEMS BY REAL TIME CONFIRMATION OF CYTOPLASMIC PENETRATION.** Amir Mor, MD PhD,1 Lauren Gatenby, B.S.,2 Emily Dzekunskas, BS,3 Linkai Zhu, Ph.D,3 Tarek K. Khader, MD,1 Julia G. Kim, MD, MPH,2 Jason M. Franasiak, MD, HCLD/ALD,1 Yiping Zhan, Ph.D,4 Emre Seli, M.D.1 1Yale School of Medicine, New Haven, CT; 2Louisiana State University, Baton Rouge, LA.

**OBJECTIVE:** Visual assessment of true cell penetration with a microinjection pipette is not always feasible due to cloudy solutions, adjacent cells, and light microscopy wavelength limitations. This is especially true for microinjection in bovine oocytes and zygotes due to their cytoplasmic opacity. We hypothesized that an increase in electrical resistance upon bovine zygote plasmatic membrane piercing can serve as a real-time tool to confirm cell penetration and embryo viability.

**DESIGN:** Experimental study.

**MATERIALS AND METHODS:** In the first part of the study, the minimal electrical resistance increase (minJR [MΩ]) that occurs when penetrating visually viable zygotes (compared to non-viable ones) was determined. Bovine zygotes were produced by in vitro fertilization. Electrical resistance of the microinjection pipette tip was measured continuously throughout the procedure. ROC analysis was performed. In the second part of the study, the ability of the minJR [MΩ] (identified in the first part of the study) to predict cell penetration and viability was tested by the microinjection of in vitro transcribed (IVT) mRNA coding for the fluorescent 'mCherry' nuclear protein into zygotes. Cleavage embryos showing nuclear fluorescence 20 hours post injection were considered viable.

**RESULTS:** ROC analysis showed minJR > 4 MΩ to identify visually viable embryos (n=67) versus non-viable ones (n=15) (97% sensitivity, 100% specificity, AUC 0.99 (CI0.95 – 1.00)). In the second part of the study, 11 zygotes had minJR > 4 MΩ at the time of IVT mtRNA injection. Seven of them (64%) cleaved and showed positive nuclear fluorescence 20 hours post injection. Eight zygotes had minJR ≤ 4 MΩ and none of them (0%) showed nuclear fluorescence 20 hours post injection.

**CONCLUSIONS:** When attempting cytoplasmic zygote microinjection, electrical resistance increase can serve as a reliable tool to confirm successful cell penetration and embryo viability, independent of optical visualization. This technology can potentially be integrated into a manual or robotic cell injection system.