Session PO2-10a - Reproductive Endocrinology, Infertility I

F-213 - A Step Towards the Automation of Intracytoplasmic Sperm Injection (ICSI): Real Time Confirmation of Oocyte Penetration by Electrical Resistance Measurement.

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Membrane Piercing,ICSI,Automation

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Abstract

Introduction: In robotic ICSI, piercing of the oolemma is determined by image processing algorithms that remain unreliable. Therefore, there is a need for an approach independent of optical visualization. We hypothesized that an increase in electrical resistance upon oocyte plasmatic membrane piercing can serve as an objective tool to confirm oocyte penetration.

Methods: Oolemma piercing with the ICSI pipette was attempted by physically advancing the pipette towards the oocyte (collected from 6-12 week-old mice) under microscopic visualization. Then, positive pressure was applied through the pipette to distend and rupture the oolemma. Electrical resistance parameters were characterized for the following: 1) TRUE MEMBRANE PIERCING: The oolemma is visually intact and, following pressure application, it distends and ruptures); 2) NEGATIVE MEMBRANE PIERCING: The oolemma is visually intact, and only the zona pellucida distends but the oolemma does not distend/rupture (following pressure application). 3) FRAGMENTED MEMBRANE: The oolemma is visually not intact (pressure was not applied since the oolemma is already ruptured). The electrical resistance of the ICSI pipette tip was measured continuously using a designated electrophysiological amplifier and median (min - max) resistances were calculated. Wilcoxon test for paired measurements was used.

Results: Significant electrical resistance increases were detected in all TRUE MEMBRANE PIERCING cases and in these cases only (n=11): 23.8 **MΩ** (17.0 - 24.3) extracellularly vs. 31.3 **MΩ** (21.0 - 130) upon cell entry ($P < 0.001$). In these cases, rupturing the membrane, by positive pressure, led to an immediate resistance drop to around the extracellular levels 23.5 **MΩ** (17.0 - 24.6). In NEGATIVE MEMBRANE PIERCING cases (n=7), no significant resistance changes were detected: 24.5 **MΩ** (23.9 - 25.3) extracellularly vs. 24.5 **MΩ** (23.4 - 25.2) during pipette advancement (in an attempt to enter the cell), and 24.5 **MΩ** (23.6 - 25.2) after pressure application (*p* was non-significant). Similarly, in FRAGMENTED MEMBRANE cases (n=12), no significant resistance changes were detected while advancing the pipette tip into the fragmented membrane space. Lastly, the baseline (extracellular) resistance did not change while touching the zona pellucida or entering the perivitelline space in any of the oocytes tested (n=30).

Conclusion: An electrical resistance increase can serve as a reliable tool to confirm oocyte penetration, independent of optical visualization. The measurements were stable, reproducible, and can be performed immediately prior to sperm injection. This method can potentially be integrated into a manual or robotic ICSI system.