Novel Platform to Assess Implantation Competency *In Vitro*

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Objective: Develop a user-friendly platform allowing clinical IVF labs to assess if embryos attach and adhere to a synthetic substrate with endometrium-like properties. This tool can then be utilized to study if variables in the pre-implantation culturing process (e.g. assisted hatching, media type, biopsy technique) positively or negatively affect implantation competency. Ultimately, utilization of this tool could lead to clinics optimizing their protocols and procedures to increase the number of live births per transfer.

Design: Instrument development and validation studies with mouse embryos

Materials and Methods: Numerous synthetic hydrogel formulations with adhesive properties were fabricated and cast into IVF culture dishes. Vitrified mouse 1-cell or blastocyst-stage embryos were warmed and cultured on the substrates at 6-6.5% CO_2 and 5% O_2 and with Gx-TL (VitroLife) media to evaluate the embryo response. An optimal formulation ("adhesive substrate" or "substrate") was identified that reliably enticed competent mouse blastocysts to attach and embed into the adhesive substrate under standard clinical IVF culturing conditions.

Appropriate volumes of adhesive substrate were added to 12-microwell square dish and ic8+ dishes (VitroLife) for culturing in a conventional, dry K-system incubator and time-lapse incubator respectively. Manufacturer-recommended volumes of culture media and oil overlaid each substrate-containing well.

Results: Both 1-cell and expanded blastocysts developed appropriately over the adhesive substrate. Hatched blastocysts consistently attached to the substrate and then spread over and into the substrate, mimicking peri-implantation events. Some trials reported a 100% "implantation" rate for hatched blastocysts.

Zona-bound, collapsed blastocysts were freed from their zona pellucida on Day 7. These newly freed embryos began attaching to the substrate within 20-40 minutes if no cell degradation was observed, and within 4-8 hours if embryonic degradation had already commenced.

Substrate-containing dishes still induced implantation-like behavior after being stored for up to 2 weeks and shipped cross-country at ambient temperature.

Conclusions: Studying implantation behavior *in vitro* by exposing blastocysts to a uterine-like substrate is currently not feasible in IVF clinics. This novel platform is cost-effective, user-friendly and works in both timelapse and traditional incubators, thus enabling IVF clinics to study how their protocols and culturing conditions can affect implantation competency. We observed that substrate-containing dishes stored and shipped at ambient temperatures performed similar to freshly fabricated substrate.

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