

Enhancing ART Efficiency: OoTrap for oocyte capture, *in vitro* maturation and fertilization

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Assisted reproductive technologies (ART) like *in vitro* maturation (IVM), fertilization (IVF), and embryo production (IVP) are essential for reproductive practices and conservation. However, the manual handling required for each step, including removing gametes and embryos from the incubator at each step, can introduce stress and compromise their quality. To prevent it, we introduce OoTrap as a single device that allows oocyte capture, IVM and IVF. OoTrap features a compact device with microwells in a dam channel, that operates in both static and perfusion-based functionality. For the fabrication of OoTrap, a two-piece mold was 3D printed using stereolithography, and devices were prepared using Polydimethylsiloxane. The two parts (perfusion channel and microwell dam) were assembled, and inlet reservoir and outlet tube were added. To evaluate the performance of OoTrap, we investigated fluid dynamics, COC entrapment, IVM and IVF efficiency. For this, follicular fluid (FF) was added into the reservoir and cell debris from FF were removed by washing the channel while keeping COCs inside. Subsequently, fertilization was performed in device without the removal of COCs. Data were analysed in R using a generalized linear mixed model, with "Treatment" as fixed and "Replicate" as random effect and a Tukey post-hoc test. A simulation study using COMSOL Multiphysics showed that hydrogen peroxide in the OoTrap can be efficiently cleared, with 83% and 98% removal after 100s, under static and perfusion (20 μ l/h) conditions, respectively. These findings suggest that OoTrap could have effective diffusion and clearance capabilities, which promote optimal culture conditions. Evaluation of trapping efficiency showed 88% entrapment (n=6). Next, we analyzed IVM efficiency under static and perfusion (20 μ l/h) conditions, with conventional IVM as control. For this, FF from 5 ovaries were added per device and left for maturation in IVM media (10% FBS, 0.1UI/mL FSH, 25 μ g/ml gentamicin 0.2mM sodium pyruvate in TCM 199) for 22-24 hours. Nuclear staining for the assessment of maturation rate (Hoechst33342, n=5) showed a higher maturation rate in the dynamic OoTrap compared to conventional IVM (70% vs 62%, respectively, p=0.05), while no differences were observed for the static OoTrap (68%. p=0.83). Immunofluorescence for the analysis of spindle morphology and chromosomal alignment (α -tubulin, Merck) showed a tendency for decreased occurrence of abnormality in the perfusion group (7%) compared to both static (22%; p=0.09) and control (25%; p= 0.067). For IVF, following IVM, media was replaced by IVF medium (Stroebech) in the devices, frozen-thawed bull sperm was washed (Sperm Wash Medium, Stroebech), added in the reservoir of OoTrap and incubated for 18-22h under 20ul/h perfusion. Presumptive zygotes were then transferred to wells containing IVC medium (ECS50 as described in Dos Santos et al, 2021) and cultured until day 9. As control, conventional IVM, IVF and IVC were performed in wells. Although OoTrap group had a significantly lower cleavage rate compared to control (63 vs 73%, respectively, p=0.0046), there was no difference in blastocyst rate (15 vs 18%, respectively, p=0.524). Next, IVM, IVF and IVC in a single device was also tested, however due to the attachment of cumulus cells in the microwells, we did not see embryo development in these devices. OoTrap's streamlined workflow and elimination of cumbersome handling steps hold promise for improving efficiency, accuracy, and success rates in ARTs.