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P-103: Automated oocyte and zygote denudation using a novel microfluidic device.

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1. Objective:

The aim of the study was to develop an automated denudation device, supervised by a computer vision algorithm, that could reduce shear stress while efficiently removing cumulus cells to allow vitrification and ICSI and for subsequent NI-PGT or metabolomics analysis.

2. Design:

Experimental comparative study using bovine and human cumulus-oocyte complexes in a novel microfluidic device.

3. Materials and Methods:

We developed a microfluidic biochip that exerts a particular fluid motion while avoiding egg entrapment within microfluidic channels. Firstly, cow cumulus oocyte complex (COCs) were used due to their size similarity with human COCs. Later human COCs were used. These were either denuded 16-20 hours post insemination for 15 min or were denuded in a second denudation run on day 3 for NI-PGT or metabolomics analysis. Alternatively, COCs were denuded for 15 min pre-insemination. COCs were classified as partially denuded if fertilization assessment, ICSI or vitrification was possible, and completely denuded if no cumulus cells remained (necessary for NI-PGT and metabolomics). Cow COCs controls were manually denuded (Stripper® pipette 145µm ID) to compare shear stress between procedures. Experiments were repeated with the use of human COCs. A Computer Vision model was developed using human COCs in order to optically assess denudation efficiency. In order to obtain meaningful performance metrics, half of the entire dataset was defined as a test set of images, with a balanced ratio of denuded and non-denuded images. The model used was a Pytorch implementation of Resnet18 with ImageNet pretrained weights

4. Results:

50 bovine COCs were microfluidically handled post insemination achieving complete (12/50) or partial (38/50) removal of the cumulus cells on day 1, while for day 3 double denudation group, 46 (92%) were completely denuded while the rest remained partially denuded. In comparison, 50/50 (100%) of manually denuded cow COC, achieved complete denudation (post insemination

group). In addition, 60% (N=10) cow COCs treated pre-insemination were partially denuded after 15 min of treatment while 100% were partially denuded after one hour of treatment.

Of 20 donated human COCs, 12 were denuded manually and 8 automatically. Those in the automatic group, were all partially denuded enough to see PNs and PBs.

The shear stress of our design was calculated to be smaller than 4.4 Pa, about ten times lower than the one applied by the manual process (~44Pa).

The deep learning algorithm was tested on 20 unseen human oocytes on day 1, with 10 true positives 9 true negatives, and 1 false negative (95% accuracy).

5. Conclusions:

Complete denudation is key to avoid DNA contamination for noninvasive PGT or metabolomics analysis, while avoiding damage to the oocyte by excessive shear force. Our automated system efficiently denude cow and human COCs with x10 less shear force without human intervention. Using a computer vision algorithm, the device could recognize degrees of denudation to subsequently treat each oocyte individually.