

## P15 - SELECTION OF THE BEST OOCYTES FOR INTRACYTOPLASMIC SPERM INJECTION (ICSI) USING APOPTOTIC ANALYSIS OF CUMULUS CELLS

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**Introduction:** We studied the apoptosis rate of the cumulus cells of individual cumulus-oocyte complex (COC), to verify a relationship with clinical outcomes, in terms of pregnancy and implantation rates. Usually oocytes are selected using morphological criteria. We tried to verify if cumulus cell apoptotic rate could be used as molecular criteria in selecting oocytes with higher implantation potentiality (1;2).

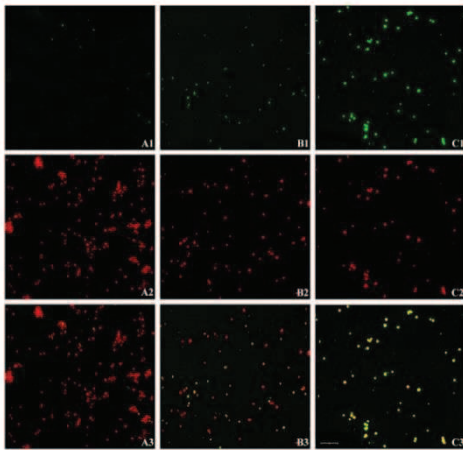
**Materials and Methods:** The study design consisted in two different trials: in the first, we investigated apoptosis rate in cumulus cells of the three selected oocytes, to be fertilized by intracytoplasmic sperm injection (ICSI); in a second trial, average apoptosis rate of the cumulus cells coming from the three selected oocytes to be fertilized by ICSI and the pooled remaining oocytes were compared, when more than 5 COCs were aspirated. In a first trial we included 22 consecutive couples undergoing ICSI cycles, 20 in a second one, for a total of 42 patients. We selected the three oocytes for (ICSI) on the basis of the morphological appearance of the cumulus, according to Veek's criteria. The cumulus cells of each COC were submitted to apoptotic assays (3). The patients were classified, on the basis of pregnancy success, in A Group (pregnant patients) and B Group (patients with negative  $\beta$ hCG).

**Results:** Both trials showed that apoptosis in the cumulus cells was remarkably lower in the A Group if compared with B Group. The apoptosis rate in the selected COCs was similar to pooled COCs for each patient, confirming that apoptosis rate in cumulus cells is characteristic for patient. Out of 22 patients involved in the first trial, 8 were pregnant (36.3% A Group) and 14 were not pregnant (B Group). In the second trial 4 of a total of 20 patients were pregnant (20%). In the first trial a total of 58 metaphase II oocytes and 56 in the second trial were studied. In the second trial 38 oocytes were pooled to compare apoptosis rate with the three selected oocytes pools. In the first trial the incidence of DNA fragmentation, evaluated by TUNEL assay (fig. 1), of the cumulus cells from individual treated oocytes, was lower in A Group than in B Group (6.7% ranging between 2.2–13.3 vs 13.19% ranging between 6.2–34.9 respectively,  $p < 0.05$ ). To confirm if DNA fragmentation was related to apoptosis process, we performed caspase-3 immunoassay in the same cells (fig. 2). Data showed a lower caspase-3 activity in cumulus cells of pregnant than in those of non-pregnant patients (5.2% ranging between 1.2–8.6 vs 11.8% ranging between 5.6–14.8,  $p < 0.05$ ). It is noteworthy to underline that pregnant patients usually exhibited, at least, one COC with a DNA fragmentation rate (TUNEL) less than 10% and caspase-3 activity rate less than 7%. Four (A Group) of 20 patients involved in the second trial were pregnant but two aborted at 8–9 weeks. The low number of pregnant patients did not allow us to have a powerful statistical analysis of apoptotic rate in cumulus cells, but it seems evident that a higher apoptotic rate in cumulus cells is associated to the pregnancy failure (B Group) and in aborted patients of A Group, ranging from 10 to 60.3%.

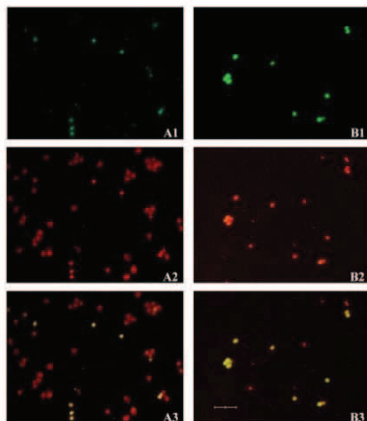
**Conclusion:** The data seem to demonstrate that apoptosis may be a marker for the selection of the best oocytes to be submitted to ICSI treatment. All pregnant patients showed a lower apoptosis rate in cumulus cells if compared with patients with pregnancy failure.

## References

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**FIGURE 1.** Apoptosis evaluation using TUNEL assay in human cumulus cells. (A1, A2, A3) A group; (B1, B2, B3) B group; (C1, C2, C3) positive control for TUNEL assay. (A1, B1, C1) fragmented DNA; (A2, B2, C2) propidium iodide staining; (A3, B3, C3) merge. Scale bar = 15  $\mu\text{m}$ .



**FIGURE 2.** Apoptosis evaluation using Cleaved caspase 3 immunofluorescence *in situ* assay in human cumulus cells. (A1, A2, A3) A group; (B1, B2, B3) B group; (A1, B1) Cleaved caspase 3; (A2, B2) propidium iodide staining; (A3, B3) merge. Scale bar = 15  $\mu\text{m}$ .