

## Apoptosis rate of human cumulus cells from single oocytes in infertile patients as criteria for the selection of oocytes to be treated by intracytoplasmic sperm injection (ICSI).

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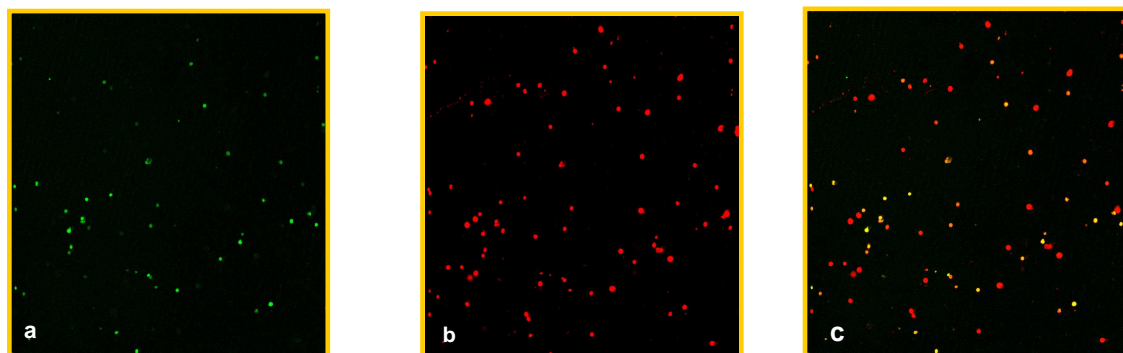
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The aim of the study was to determine if the apoptosis rate of cumulus cells derived from human oocytes can be associated with better results in terms of fertilization, embryo cleavage, pregnancy and implantation rate, in patients undergoing ICSI procedure for male factors.

In our previous papers we have demonstrated that the apoptosis rate in cumulus cells is strictly related to the oocyte competence in terms of nuclear and cytoplasmic maturation. The recent Italian law, which limits the number of oocytes to be treated, is actually improving research on gamete selection. Usually gametes, in particular oocytes, are selected on the basis of morphological criteria. Our objective, has been to find a new way for oocyte selection, using molecular tests investigating apoptosis of the cumulus cells. The cumulus-oocyte complexes (COCs) were treated soon after oocyte aspiration following hormonal ovarian stimulation. All the COCs showing good morphological aspect were treated by hyaluronidase. The degree of apoptosis of cumulus cells for each single metaphase II (MII) oocyte was evaluated using TUNEL assay and cleaved-caspase 3 immunoassay. Only 3 oocytes, to be treated by ICSI, were investigated singularly, the others were analyzed as a pool. All the patients who were pregnant after ICSI treatment showed a lower apoptosis rate, evidenced in both methods. In particular we found that all 3 oocytes showed an apoptosis rate less than 15% in the pregnant patients. In the patients who were not pregnant we found that at least one oocyte shows an apoptotic rate in its own cumulus cells of more than 15%.

The data seems to demonstrate that a lower apoptosis rate in the cumulus cells is associated with the oocyte quality in terms of capacity to be fertilized, and undergoing embryogenesis culminates in an increased implantation and pregnancy rate. This molecular criteria could be used together with morphological criteria of the COCs for the selection of the 3 best oocytes to be treated by ICSI.



Sezioni ottiche equatoriali, osservate con microscopio confocale laser:

**Fig. a:** reazione TdT

**Fig. b:** controcolorazione con propidio ioduro

**Fig. c:** sommatoria (a+b)