EVALUATION OF EQUINE ZONA PELLUCIDA IN POLARIZED LIGHT MICROSCOPY

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ARTs (Assisted Reproduction Technologies) are essential means for the preservation of valuable genetic resources; furthermore, they are powerful tools which often provide clinical answers for hypofertile patients recovering.
The observation of female gametes immediately after collection or cryopreservation, with polarized light microscopy, could be a useful technique for the investigation of effective gamete quality without causing cell damage.
Purpose of this study was to evaluate the correlation between parameters obtained from the measurement of oocyte birefringent structures (area, retardance and thickness of the inner layer of zona pellucida (IL-ZP), and total thickness of ZP) and the parthenogenetic activation response of the cells, in order to identify markers of good gamete quality in horses.

199 COCs (Cumulus-Oocyte Complex) were collected from 40 ovaries obtained from a local slaughterhouse. After collection, the gametes were incubated in maturation medium and then, denuded of cumulus cells, 51 of them were analyzed with PLM (Polarized Light Microscopy) after 36 h of IVM (In Vitro Maturation). Later, 28 mature oocytes were submitted to parthenogenetic activation, transferred in SOF for 3-6 days and daily inspected in order to evaluate the progression of activation.

It was obtained an average of cleaved oocytes per work session equal to 80,24 ± 5,36%.
The ZP birefringent proprieties were estimated and then correlated to the activation outcome.
The data obtained showed that the thickness of ZP was significantly increased in immature oocytes compared to mature oocytes (20,78 ± 3,12 µm vs 18,85 ± 2,38 µm, $p<0,05$).
The comparison between parthenogenetically activated and non-activated oocytes showed a significantly increase of IL-ZP thickness in parthenogenetically activated oocytes compared to the parthenogenetically non-activated (4,79 ± 1,00 µm vs 4,18 ± 0,30 µm, $p<0,05$).
Furthermore, it has been observed an increasing trend for IL-ZP area values in the parthenogenetically activated oocytes compared to the parthenogenetically non-activated (2456,00 ± 448,76 µm2 vs 2220,00 ± 113,70 µm2).
As for women, the PLM in equine oocytes allows the non-invasive observation of zona pellucida, a birefrigent oocyte structure, considered as marker of gamete quality. The application of this method could permit to direct towards subsequent ARTs just the cells with a high quality level, having an increase of fertilization percentage. In order to pursue this aim it could be necessary to increase the number of analyzed oocytes to confirm and enhance the results of this preliminary work.

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Riproduzione equina
horse, oocyte, polscope