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Award Winners

A-7

Four hours or more preincubated oocytes in the simple medium provide low transcript levels of maternal effect genes for the embryos

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Background: Preincubation is the temporary cultivation of oocytes at 37°C and 5-6% of CO2 before ART procedures. There is not any explanation regarding a standard preincubation time in ART laboratory guidelines and it is dependent completely on the laboratory workload. Myo-inositol as the most important form of inositol, is involved in several systemic processes and its antioxidant action has been suggested recently. The study aimed to evaluate the effect of oocyte preincubation time and also myo-inositol as a medium supplement on the oocyte Zar1, Nlrp5, Npm2 transcript levels as well as the fertilization and first cleavage rates.

Objective: The study aimed to evaluate the effect of oocyte preincubation time and also myo-inositol as a medium supplement on the oocyte Zar1, Nlrp5, Npm2 transcript levels as well as the fertilization and first cleavage rates.

Materials and Methods: Cumulus Oocyte Complexes which were retrieved from superovulated NMRI mices were divided randomly in five experimental groups: (1) control (2) 4 hours preincubation in simple medium (3) 4 hours preincubation in 20 mmol/l of myo-inositol supplemented medium (4) 8 hours preincubation in simple medium (5) 8 hours preincubation in 20 mmol/l of myo-inositol supplemented medium. COCs were denuded and transcript levels of Zar1, Nlrp5 and Npm2, selected by bioinformatics, were assessed by real time qPCR method. 2PN and 2-cell rates were analyzed following oocytes and sperms co-incubation. One-way ANOVA and Kruskal-Wallis were respectively used for parametric and nonparametric variables. Statistical significance was defined as P-value ≤ 0.05.

Results: Zar1 (1-fold vs 0.4-fold) and Npm2 (1-fold vs 0.2-fold) transcript levels, as well as 2PN (84.64 ± 4.02 vs 78.90 ± 1.11) and 2-cell rates (79.58 ± 1.45 vs 59.85 ± 9.44) were reduced after 4 h of preincubation time in the simple medium compared to the control group. While Nlrp5 transcript level (1-fold vs 0.07-fold) was significantly decreased following 8 h of preincubation time in the simple medium ($p \square 0.001$). Addition of myo-inositol to the culture medium could ameliorate maternal effect genes levels and fertilization and first cleavage rates in the oocytes preincubated for 4 and 8 hours ($p \square 0.001$).

Conclusion: However it has not found a clear boundary between optimal and non-optimal oocyte preincubation time, our findings addressed that 4 h or more preincubation time can influence the oocyte mRNA storage and ultimately leads to reduce oocyte fertilization and first cleavage rates. Besides, medium supplementation with myo-inositol could preserve the mRNAs inherited to the embryos and consequently improve fertilization and first cleavage developmental rates.

Key words: *Oocyte preincubation time, Maternal effect genes, Fertilization potential, First cleavage rate, Myo-inositol supplement.*

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