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The effect of cumulus cells on domestic cat oocytes during in vitro maturation and in vitro fertilization

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Cumulus cells (CC) are important for oocytes growth, maturation and fertilization. They supply the oocytes with nutrients and connect them with external word, but during cryopreservation, CC around the oocyte decrease the permeation of the cryoprotectant into the oocytes, affecting the results [1]. It has been shown on porcine and bovine oocytes that the co-culture of denuded oocytes (DO) (freed from CC) with loose CC and cumulus oocytes complexes (COC) has a positive effect on their maturation rate and the cleavage rate after fertilization. Studies performed on cat oocytes [2, 3] showed positive influence of some co-culture systems on resumption of meiosis and fertilization. The aim of the present study was to evaluate the effect of the co-culture of denuded oocytes with CC or COC on the results of in vitro maturation (IVM) (experiment 1) and in vitro fertilization (IVF) (experiment 2). Immature oocytes were collected from ovaries of domestic cat after a routine ovariectomy. Only the oocytes with dark, homogenous cytoplasm, surrounded by several layers of compacted CC were selected for the study. In experiment 1, oocytes were in vitro matured for 24 h in four groups: (i) DO, (ii) DO co-cultures with CC, (iii) DO co-cultured with COC, (iv) COC as a control group. Individual oocytes were denuded mechanically with the use of the Stipper® device. In experiment 2, COC were in vitro matured for 24 h and then oocytes were randomly divided into four groups analogous to that in experiment 1 and then in vitro fertilized with the use of sperm collected from adult tom cat epididymis. After 18 h of co-incubation presumptive zygotes were moved to embryo culture medium and cultured up to 7 days. In the end of each experiment oocytes/embryos were fixed, stained in Hoechst 33342 (2 µg/ml solution) and observed under the epifluorescence microscope in order to assess oocytes maturation stage or embryo development. Data were compared with χ2 test, probabilities of less than 0.05 were considered statistically significant. In experiment 1, meiotic competence, described by the percentage of oocytes that reached MII stage in the course of IVM, of denuded oocytes decreased significantly in all experimental groups, when compared to the control group. Maturation rate amounted 45% (30/66), 24% (14/59), 43% (22/51) and 76% (46/57) (p<0.05), in a group (i), (ii), (iii) and (iv), respectively. In experiment 2, embryos up to morula stage developed in all experimental groups. DO and oocytes cultured with COC during fertilization showed lower cleavage rate – 36% (9/25) and 25% (3/12) than those co-cultured with loose CC and from the control group 43% (12/28) and 42% (16/38), respectively. Results of this study show that cumulus cells connected with oocyte into an cumulus oocyte complex are irreplaceable in the process of maturation of domestic cat oocyte, but addition of loose CC might be useful in the process of IVF.

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