ABSTRACTS

ISCFR 2012

July 26-29, Whistler, Canada

7th International Symposium on Canine and Feline Reproduction

In a joint meeting with

EVSSAR 2012

15th Congress of the European Veterinary Society for Small Animal Reproduction

Editors: Gary England, Michelle Kutzler, Pierre Comizzoli, Wojciech Nizanski, Tom Rijsselaere and Patrick Concannon

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Characterization of mitochondrial and actin patterns in cat oocytes and blastocysts

González, R1; Gómez, MC2; Pope, CE2 and Brandt, YCB1

1Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden and 2 Audubon Nature Institute Center for Research of Endangered Species, New Orleans, LA 70131, USA.

rrraquelgh2@hotmail.com; raquel.gonzalez.herrero@slu.se

OBJECTIVES AND METHODS: The spatial relocation of mitochondria throughout oocyte maturation and the actin polymerization into microfilaments are important for meiosis progression and subsequent embryo development. Both parameters are useful markers of cytoplasmic maturation (1), but no information is available regarding actin and mitochondria distribution in cat oocytes and embryos. Therefore, the purpose of this study was to: a) assess cytoplasmic characteristics of the oocyte by mitochondria and actin staining in immature (germinal vesicle stage) and in vitro/in vivo matured (metaphase II) cat oocytes and; b) characterize mitochondria and actin distribution in in vitro produced blastocysts. Oocytes were recovered by slicing ovaries after ovariohysterectomy in collection medium and incubated for 24 h in maturation medium. Afterwards, fertilization and in vitro culture procedures were done as explained previously (2). Additionally, in vivo matured oocytes were collected by laparoscopy from two superovulated females as described (3). Actin microfilaments and mitochondrial patterns were evaluated by laser scanning confocal microscopy using Alexa Fluor-488 Phalloidin and MitoTracker Orange, respectively. Hoechst 33342 was used for DNA staining. Data were analyzed as categorical variables using Pearson chi-squared test and Cramér’s V coefficient.

RESULTS: In oocytes, cortical microfilaments showed a similar pattern (P > 0.05). The presence of transzonal cumulus cell projections was identified by actin staining and differed with the source of oocytes (P < 0.001). Immature oocytes (n = 54) were characterized by the presence of a complete network of transzonal projections (78%), whereas they were absent in 96% of the in vitro matured oocytes (n = 76). In contrast, 76% of the in vivo matured oocytes (n = 21) presented remnants of cumulus cells through the zona. There was no clear relocation and aggregation of active mitochondria during oocyte maturation. Mitochondria were localized in the periphery of the oocyte in 84% of the immature (n = 54), 87% of in vitro (n = 73) or 71 % of in vivo (n = 21) matured oocytes. In vitro matured oocytes (12%) presented a semiperipheral pattern while 29% of in vivo matured oocytes had some active mitochondria in the central part of the ooplasm (P < 0.001).

A total of 71 blastocysts were analyzed to assess actin cytoskeleton. Most blastocysts had good quality cytoskeleton (grade I, 63%), 28% showed fair quality (grade II) and 9% had lost most of their cellular integrity (grade III).

For mitochondria staining, 50 blastocysts were analyzed. Of them, 52% were classified as having even distribution (grade I), 36% had intermediate (grade II) and 12% had very heterogeneous (grade III) distribution of mitochondria. In blastocysts, actin and mitochondrial patterns were related (P < 0.001).

CONCLUSION: The present study represents the original description of actin and mitochondrial patterns in cat oocytes and embryos. Transzonal cumulus cell projections were more abundant in immature oocytes than in matured oocytes. A relocation of mitochondria throughout meiosis was not clearly observed. However, most in vitro produced blastocysts were of good quality, according to their actin cytoskeleton integrity and mitochondria distribution. The functional significance of mitochondria distribution in cat oocytes in relation to their developmental competence requires further research.

Funded by Carl Tryggers Stiftelse för Vetenskaplig Forskning and Formas, Stockholm, Sweden.