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“Development on the margin”

Effects of Ascorbic Acid and Glutathione on *in Vitro* Maturation of New Zealand White and Baladi Black Rabbit Oocytes

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Abstract

The objective of this study was to evaluate the effects of level of some antioxidants such as ascorbic acid (AA) and reduced glutathione (GSH), on *in vitro* maturation rate of New Zealand White (NZW) and Baladi Black (BB) rabbit oocytes. Twenty Oocytes were immediately subjected after collection to slicing technique. Only compact cumulus oocytes (COCs) were used. Five types of maturation medium were used included TCM-199 supplemented with 2 or 3 mM of AA and 0.5 or 1 mM of GSH as well as control medium (without supplementation). The medium was initially equilibrated in CO₂ incubator (5 % CO₂, at 38°C with saturated humidity) for 60 minutes, then the oocytes were cultured in the medium under the same conditions for 20 h as a maturation period. Oocytes were fixed (with 3ethanol:1glacialacid), stained (with 1 % orcein in 45 % acetic acid), and microscopically examined. The results revealed that supplementation with 2 mM AA yielded the highest percentage of NZW oocytes arrested at M-II stage (70.6 %), but did not differ significantly from that achieved with the supplemental level of 0.5 mM GSH (67.3 %). Supplementation with 3 mM AA gave significantly higher percentage (62.5 %), and supplementation with 1 mM GSH exhibited significantly similar percentage (57.5 %) of NZW oocytes arrested at M-II as compared to that of their respective control medium (58.7 %). As for BB oocytes, supplementation with 3 mM AA yielded the highest percentage of oocytes arrested at M-II stage (79.4 %), but did not differ significantly from that (77.5 %) obtained with the low AA supplemental level (2 mM). Supplementation with either level of GSH (0.5 and 1 mM) failed to improve percentage of BB oocytes arrested at M-II as compared to that achieved with their respective control medium (58.3 and 64.1 vs. 71.8 %, respectively). Overall percentage of oocytes arrested at M-II was higher in BB than that in NZW does (70.1 vs. 63.0 %). The results suggest that supplementation of TCM-199 with AA had a beneficial effect on the *in vitro* maturation of rabbit oocytes and that can be achieved at level of 2 mM for NZW and 3 mM for BB oocytes.

Keywords: Ascorbic acid, Baladi, glutathione, *in vitro* maturation, NZW, rabbit